ARTICLE

Increased serum amiodarone concentration in hypertriglyceridemic patients: Effects of drug distribution to serum lipoproteins

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Abstract

Amiodarone and its main metabolite, desethylamiodarone (DEA), are highly distributed to serum lipoproteins such as very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), which are the carriers of triglyceride and cholesterol. This study aimed to investigate the association of serum concentrations of amiodarone and DEA with the levels of serum lipids in terms of drug distribution to lipoprotein fractions in patients with hyperlipidemia. Total serum concentrations of amiodarone and DEA were examined in 116 patients receiving amiodarone for tachyarrhythmias. The concentration-to-dose (C/D) ratio of amiodarone positively correlated with the level of serum triglyceride ($r_s = 0.541$, $p < 0.001$) and was higher in the hypertriglyceridemic state than in normotriglyceridemic state (479 ± 211 vs. 320 ± 161, $p < 0.001$). No correlation was found between the C/D ratio of DEA and serum triglyceride levels ($r_s = 0.272$), although higher values were observed in the hypertriglyceridemic state (322 ± 125 vs. 285 ± 143, $p < 0.001$). In the hypertriglyceridemic state, the distribution of amiodarone increased in LDL/VLDL fraction and decreased in high-density lipoprotein and albumin fractions. The ratio of serum amiodarone to serum DEA, a metabolic ratio of amiodarone, positively correlated with serum triglyceride levels ($r_s = 0.572$, $p < 0.001$) and was higher in the hypertriglyceridemic state, suggesting that amiodarone metabolism decreased in hyperlipidemia. The results of this study reveal that serum concentrations of amiodarone increase in the hypertriglyceridemic state through the increased lipoprotein-binding and decreased metabolism of amiodarone.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Lipoproteins can carry not only serum lipids but also certain lipophilic compounds such as amiodarone. Changes in the lipoprotein-binding of amiodarone

may lead to highly variable pharmacokinetics and poor concentration–effect relationships of the drug.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study addressed the association between serum amiodarone concentration and serum lipid levels in patients with arrhythmia, as well as the lipoprotein-binding and metabolism of amiodarone in the hyperlipidemic state.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Serum amiodarone concentration is increased in patients with hypertriglyceridemia and it is positively correlated with serum triglyceride levels. These results are attributable to an increase in the circulating lipoprotein-bound form of amiodarone and decreased metabolism of the drug in the hypertriglyceridemic state.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Changes in the lipoprotein-binding of amiodarone can help explain differences in the pharmacokinetics and pharmacodynamics of amiodarone related to normo-triglyceridemic and hypertriglyceridemic states.

INTRODUCTION

Amiodarone is a class III antiarrhythmic drug commonly used to treat atrial fibrillation and ventricular arrhythmias.[1,2] Amiodarone therapy must be terminated when adverse events occur; these are typically observed in greater than 50% of patients receiving long-term amiodarone treatment.[3,4] Although therapeutic drug monitoring of amiodarone is an approach used to avoid severe adverse effects, convincing data on the predictive blood concentration range for clinical effects/toxicity of amiodarone are currently lacking.[5,6] Thus, there remains a need to better understand the pharmacokinetic characteristics of amiodarone, including its delivery to target tissues.

Amiodarone is predominantly metabolized to an active metabolite, desethylamiodarone (DEA), by cytochrome P450 (CYP) 3A4 and CYP2C8.[7] Both amiodarone and DEA, which possess highly lipophilic properties, show unique and highly variable pharmacokinetics with a large distribution volume and long elimination half-life.[8–10] Amiodarone accumulates in the liver, fat, and lungs with high tissue/plasma ratios (>100) when used long term.[11] Because circulating amiodarone is known to bind at a high rate to serum proteins, such as albumin and lipoproteins,[12] changes in the tissue distribution and protein binding of amiodarone may result in highly variable pharmacokinetics.

Lipoproteins, which transport serum lipids such as triglyceride and cholesterol in the aqueous environments of the blood stream and lymphatic fluid, can be classified as chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Lipoproteins are currently the subject of research because they can carry certain lipophilic compounds such as fat-soluble vitamins and drugs.[13] Amiodarone and DEA are also distributed extensively to serum lipoproteins, such as VLDL and LDL.[14,15] In previous research, the distribution volume of amiodarone was markedly reduced in experimental hyperlipidemic rats due to a decrease in distribution to peripheral tissues.[16,17] Additionally, VLDL/LDL-binding drugs are known to be transported into tissues via LDL receptor (LDLR)-mediated uptake in LDLR-overexpressing mice.[15] Thus, altered lipoprotein-associated drug distribution may play important roles in drug metabolism, drug efficacy, and adverse drug effects.

In the present study, we investigated the effects of serum lipid levels on total serum concentrations of amiodarone and DEA in patients with arrhythmia. Specifically, we assessed the influence of lipoprotein-binding amiodarone on changes to the serum concentration and hepatic metabolism of amiodarone in the hyperlipidemic state.

METHODS

Patients and data collection

In total, 116 patients receiving oral amiodarone for supraventricular and ventricular tachyarrhythmias were included in the study, which was conducted at the University of Tsukuba Hospital (Table 1). Patients receiving concomitant drugs, including CYP3A4 inducers (rifampicin, phenytoin, phenobarbital, and carbamazepine), CYP3A4 inhibitors (anti-HIV agents, azole antifungal agents, macrolide antibiotics, verapamil, and diltiazem), or CYP2C8 inhibitors (clopidogrel), were excluded from the study. The following data were retrospectively collected from the medical records of the patients: age; body weight; body mass index (BMI); dose and duration of amiodarone administration; concomitantly received drugs; laboratory data, such as serum aspartate aminotransferase, alanine aminotransferase,

TABLE 1 Patient characteristics

Characteristics	Overall	Normotriglyceridemia	Hypertriglyceridemia
Number of patients	116	91	50
Sex, male/female	81/35	61/30	38/12
Age, years	62 ± 12	63 ± 12	60 ± 13
Body weight, kg	60 ± 13	58 ± 12	65 ± 14
Body mass index, kg/m^2	22.8 ± 3.5	22.2 ± 2.9	23.9 ± 3.9
Diagnosis			
Supraventricular tachyarrhythmias (AF/AT/AFL)	53 (48/4/1)	41 (37/4/0)	22 (19/2/1)
Ventricular tachyarrhythmias (VF/VT/PVC)	63 (3/51/9)	50 (2/42/6)	28 (2/21/5)
Concomitant lipid lowering drugs			
Statins	51	36	26
Ethyl icosapentate	4	4	1
Ezetimibe	2	1	1
Number of measurements	398	241	157
Number of measurements per person	2 (1–74)	1 (1–52)	1 (1–22)
Liver and kidney function			
Aspartate aminotransferase (U/L)	27 ± 20	29 ± 24	23 ± 9
Alanine aminotransferase (U/L)	24 ± 20	26 ± 25	22 ± 9
Creatinine clearance (ml/min)	67 ± 30	63 ± 31	74 ± 27
Serum lipids levels			
Triglyceride (mg/dl)	153 ± 127	97 ± 29	239 ± 164
Total cholesterol (mg/dl)	195 ± 39	185 ± 39	210 ± 35
Serum albumin (g/dl)	4.1 ± 0.4	4.0 ± 0.5	4.2 ± 0.3
Amiodarone dose (mg/kg)	2.2 ± 1.0	2.3 ± 1.0	2.0 ± 0.9
Duration of therapy (days)	691 (4–8051)	779 (10–8051)	499 (4–4486)
Serum amiodarone concentration (ng/ml)	701 (79–2406)	631 (79–2406)	827 (203–2244)
Serum desethylamiodarone concentration (ng/ml)	588 (106–1783)	585 (106–1565)	601 (163–1783)
Amiodarone/desethylamiodarone ratio	1.30 ± 0.42	1.15 ± 0.32	1.52 ± 0.45

Note: Data are presented as the number of patients, mean ± SD, or median (range) depending on the variable. The amiodarone/desethylamiodarone ratio represents the serum concentration ratio of amiodarone to desethylamiodarone.

Abbreviations: AF, atrial fibrillation; AT, atrial tachycardia; AFL, atrial flutter; VF, ventricular fibrillation; VT, ventricular tachycardia; PVC, premature ventricular contraction.

creatinine, albumin, triglyceride, and total cholesterol, and the total serum concentrations of amiodarone and DEA. The concentration-to-dose (C/D) ratios for amiodarone and DEA were defined as serum concentration/dose per body weight. The time required to reach steady-state concentrations of amiodarone and DEA was at least 6 months.[18] Hypertriglyceridemia and hypercholesterolemia were categorized as serum triglyceride levels greater than or equal to 150 mg/dl and total cholesterol levels greater than or equal to 220 mg/dl, respectively.[19] No patient co-existed with liver cirrhosis; however, some patients had suffered from mild congestive liver ($n = 4$), fatty liver ($n = 4$), hepatitis C ($n = 4$), and alcoholic liver injury ($n = 1$).

This study was approved by the Ethics Committee of the University of Tsukuba Hospital. For data collection in the observational study, the informed consent of patients was obtained based on a waived requirement (opt-out style). Written informed consent was obtained from the patients to measure the contents of amiodarone and DEA in each serum fraction.

Chemicals

Amiodarone hydrochloride, DEA hydrochloride, and the internal standards (amiodarone-d$_4$ hydrochloride and desethylamiodarone-d$_4$ hydrochloride) were purchased from Toronto Research Chemicals (Toronto, Canada). All other chemicals were of commercially available analytical reagent grade.

Preparation of spiked serum samples

Spiked serum samples were prepared using serum samples collected from six patients not receiving amiodarone. These serum samples were then spiked with amiodarone and DEA at concentrations of 500 ng/ml with a methanol content of 0.1%. The spiked serum samples were incubated at 37°C for 60 min and then subsequently cooled to 4°C for 2 h.

Fractionation of lipoproteins and albumin from patient serum

To determine the contents of amiodarone and DEA in lipoproteins and albumin fractions, we used 18 serum samples from 13 patients receiving oral amiodarone and six spiked serum samples. The LDL/VLDL, HDL, and albumin fractions were extracted from 100 µl of serum using LDL/VLDL and HDL Purification Kits (Cell Biolabs, San Diego, CA) according to the manufacturer's protocol. Briefly, separation was based on precipitation with the addition of dextran sulfate and differential centrifugation. LDL/VLDL was precipitated by centrifugation with dextran at 9000 g for 10 min; the supernatant was separated into HDL and albumin fractions by centrifugation with dextran at 18,000 g for 30 min. Each separated serum fraction for LDL/VLDL, HDL, and albumin was confirmed by determining LDL and HDL cholesterol, apolipoprotein B and A-1 levels, and albumin (Table S1). The contents of amiodarone and DEA in each fraction were expressed as the fractional percentage recoveries relative to overall levels in the serum.

Liquid-chromatography tandem mass spectrometry apparatus and analytical conditions

Liquid-chromatography tandem mass spectrometry (LC-MS/MS) analyses were performed using an API 3200 LC-MS/MS system coupled to ekspert ultra LC 100 (AB Sciex, Tokyo, Japan). An octadecylsilyl silica (ODS) column (TSKgel ODS-100Z; 2.0 mm I.D. ×50 mm; TOSOH, Tokyo, Japan) was used and maintained at ambient temperature. The gradient elution method used for chromatographic separation is shown in Table S2.

    The mass spectrometer was operated in the electrospray positive ionization mode, and system control and data acquisition were performed using Analyst software version 1.6.1 (AB Sciex). The settings were set as follows: ion spray source temperature at 500°C, ion spray voltage at 5500 V, curtain gas at 10, ion source gas 1 at 80, ion source gas 2 at 60, and collision gas at 4. Sample analysis was performed in the multiple reaction monitoring mode; the optimized conditions for monitoring the m/z of parent and product ions are shown in Table S3.

Assay procedures

Aliquots of serum samples (100 µl) or each serum fraction were deproteinized with 300 µl of acetonitrile containing the internal standards: 500-ng/ml amiodarone-d_4 and 500-ng/ml desethylamiodarone-d_4. For each serum fraction, the mixture was further incubated at 60°C for 10 min and then vortexed for 30 min. After centrifugation at 18,000 g for 10 min, 10 µl of supernatant was injected into the LC-MS/MS system. The quantification limits were 5 ng/ml each for amiodarone and DEA. The coefficients of variation for intra- and interday assays were 2.7%–4.6% and 3.0%–6.5% for amiodarone, and 2.1%–9.3% and 3.2%–9.5% for DEA, respectively. The linearity of amiodarone and DEA contents in each serum fraction to the total serum concentration was confirmed at 50–2500 ng/ml (Figure S1).

Statistical analyses

Data were analyzed with SPSS Statistics 25 (IBM, Armonk, NY, USA). Continuous variables in hyperlipidemic and normolipidemic groups were compared using a Mann–Whitney U test. Continuous variables among serum fractions were compared using a Mann–Whitney U test with Bonferroni's correction following a Kruskal–Wallis test. Correlations between variables were examined by calculating Spearman's rank correlation coefficients (r_s) or Pearson's correlation coefficients (r) after assessing for normality of data distribution. Proportions were compared using a χ^2 test or Fisher's exact probability test. The p values less than 0.05 were considered statistically significant. Correlations were considered strong when the values correlated well (r_s or r, >0.6 or <−0.6), with p values less than 0.05. The r_s or r values between 0.5 and 0.6 or between −0.5 and −0.6, with p values less than 0.05, were considered to represent a moderate correlation.

RESULTS

Effects of serum lipids on serum concentrations of amiodarone and DEA

In total, 398 measurement data were collected from 116 patients receiving oral amiodarone. Demographic data, clinical characteristics, and serum concentrations of

amiodarone and DEA are summarized in Table 1. Of the 398 data, 157 and 114 were from the hypertriglyceridemic and hypercholesterolemic states, respectively. Correlation matrix of serum levels of triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol and albumin, and BMI are shown in Table S4.

The C/D ratio of amiodarone was positively correlated with serum triglyceride levels ($r_s = 0.541$; Figure 1a) and higher values were detected in the hypertriglyceridemic state than were found in the normotriglyceridemic state (479 ± 211 vs. 320 ± 161, $p < 0.001$; Figure 2a). No correlation was found between the C/D ratio of DEA and serum triglyceride levels ($r_s = 0.272$; Figure 1c), although higher values were obtained in the hypertriglyceridemic state

(322 ± 125 vs. 285 ± 143, $p < 0.001$; Figure 2c). The ratio of serum amiodarone to DEA (amiodarone/DEA) was positively correlated with serum triglyceride levels ($r_s = 0.572$; Figure 1e) and higher values were detected in the hypertriglyceridemic state (1.52 ± 0.45 vs. 1.15 ± 0.32, $p < 0.001$; Figure 2e). Similar results were found before ($n = 71$) and at steady-state ($n = 327$): 377 ± 200 vs. 190 ± 93, $p < 0.001$, and 507 ± 205 vs. 345 ± 159, $p < 0.001$, for C/D ratio of amiodarone; 1.57 ± 0.48 vs. 1.22 ± 0.41, $p < 0.001$, and 1.51 ± 0.45 vs. 1.14 ± 0.30, $p < 0.001$, for amiodarone/ DEA ratio, respectively.

The C/D ratios for both amiodarone and DEA, as well as the amiodarone/DEA ratio were not correlated with serum cholesterol levels ($r_s = 0.251$, $r_s = 0.200$, and $r_s = 0.167$,
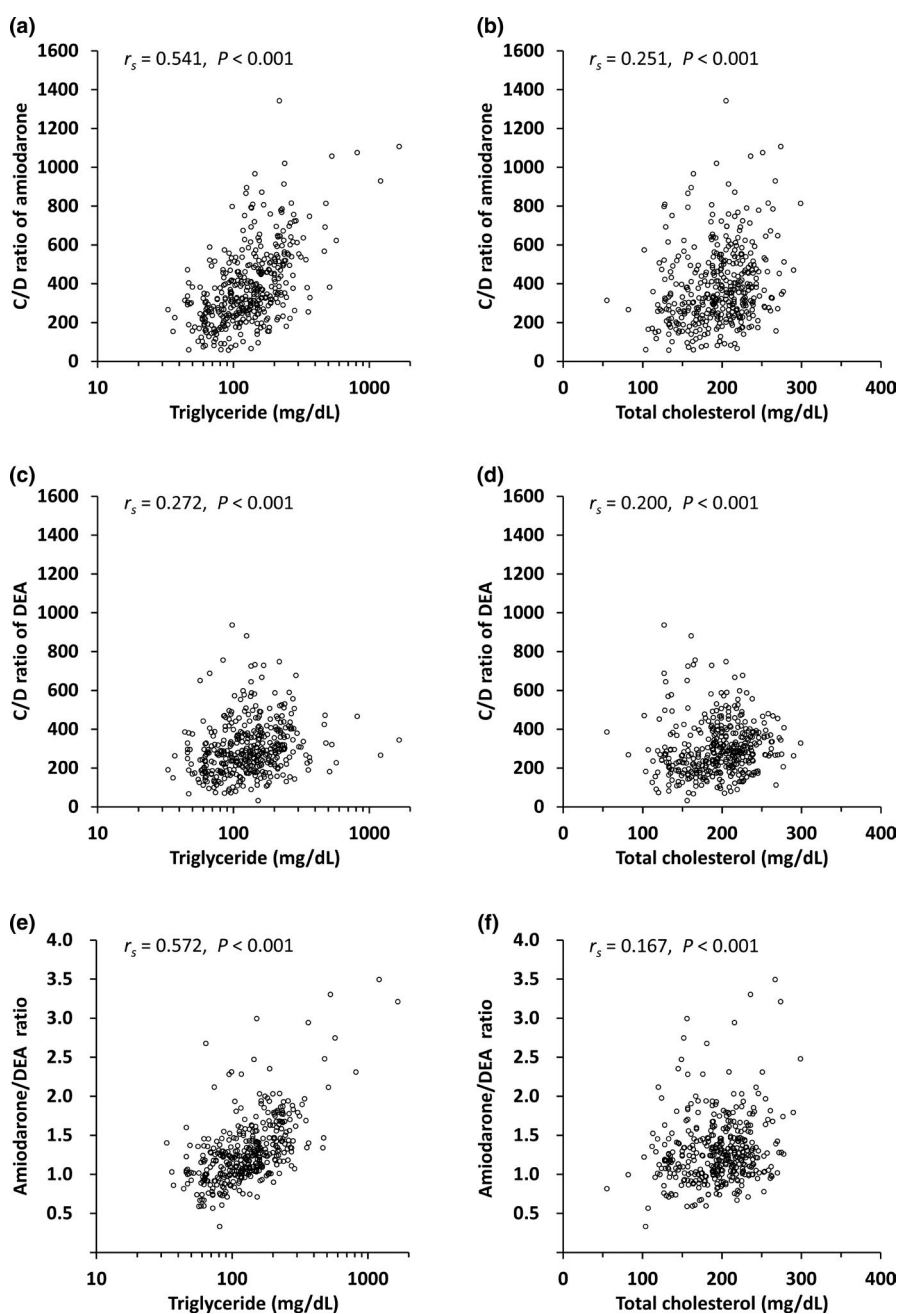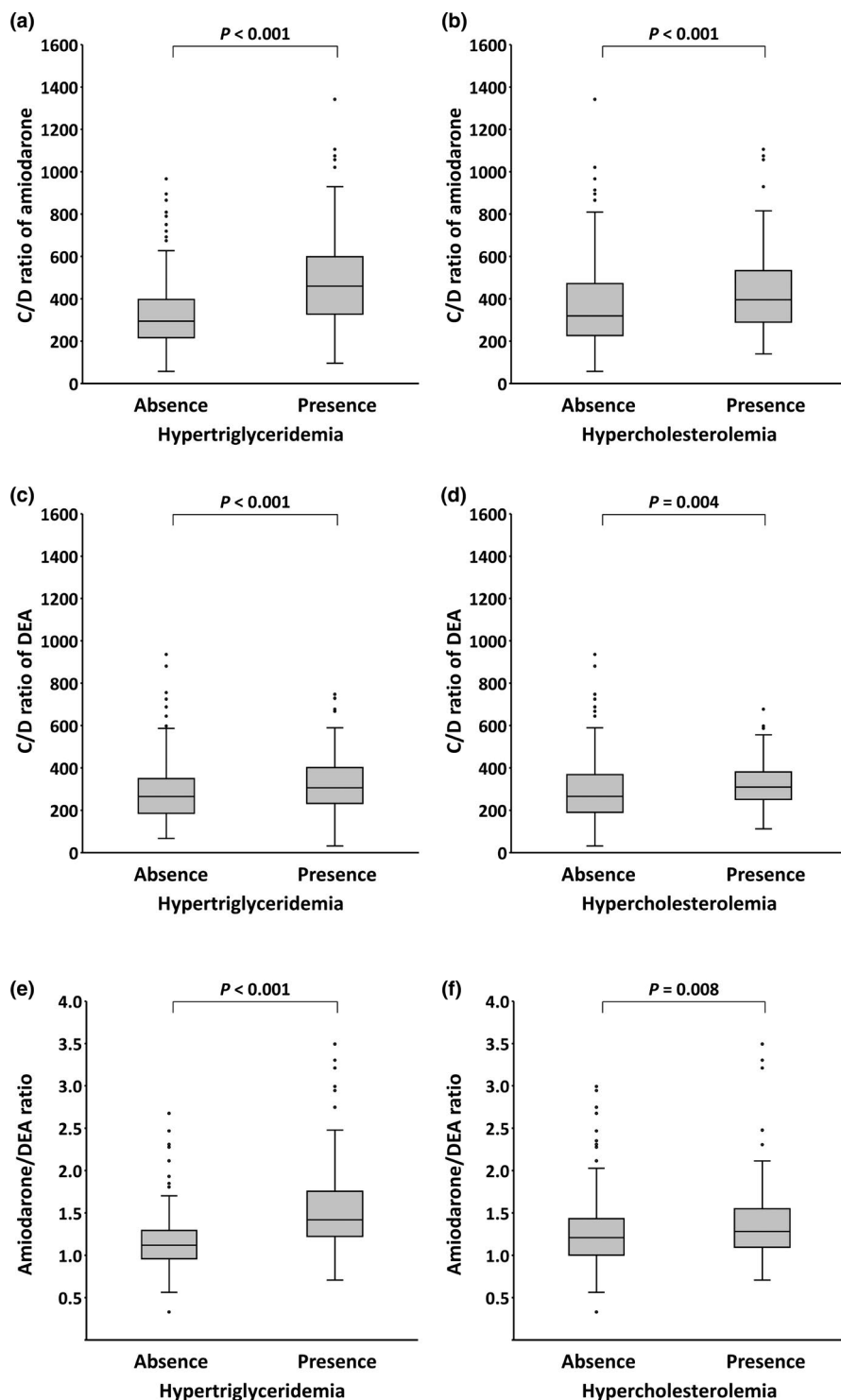

FIGURE 1 Effect of serum levels of triglyceride and total cholesterol on the concentration-to-dose (C/D) ratios of amiodarone (a, b) and desethylamiodarone (DEA; c, d), and the serum concentration ratio of amiodarone to DEA (amiodarone/DEA ratio; e, f)

respectively; Figure 1b,d,f), although significantly higher values were observed in the hypercholesterolemic state relative to those in the normocholesterolemic state (amiodarone: 436 ± 200 vs. 362 ± 194, $p < 0.001$; DEA: 317 ± 110 vs. 293 ± 146, $p = 0.004$; amiodarone/DEA ratio: 1.39 ± 0.46 vs. 1.26 ± 0.40, $p = 0.008$; Figure 2b,d,f). The amiodarone C/D ratio was not correlated with BMI ($r_s = 0.256$). Significantly higher amiodarone C/D ratios in the hypertriglyceridemic state were observed in both BMI less than 25 kg/m²

$(468 \pm 203$ vs. 317 ± 181, $p < 0.001$; data not shown) and BMI greater than or equal to 25 kg/m² (491 ± 218 vs. 330 ± 97, $p < 0.001$). Similar results for amiodarone C/D ratio were found in both men (489 ± 223 vs. 334 ± 166, $p < 0.001$) and women (431 ± 123 vs. 284 ± 139, $p < 0.001$).

Lower total cholesterol levels and higher triglyceride levels were found in the patients with co-administration of statins relative to those without co-administration (cholesterol: 176 ± 38 vs. 205 ± 36, $p < 0.001$; triglyceride:
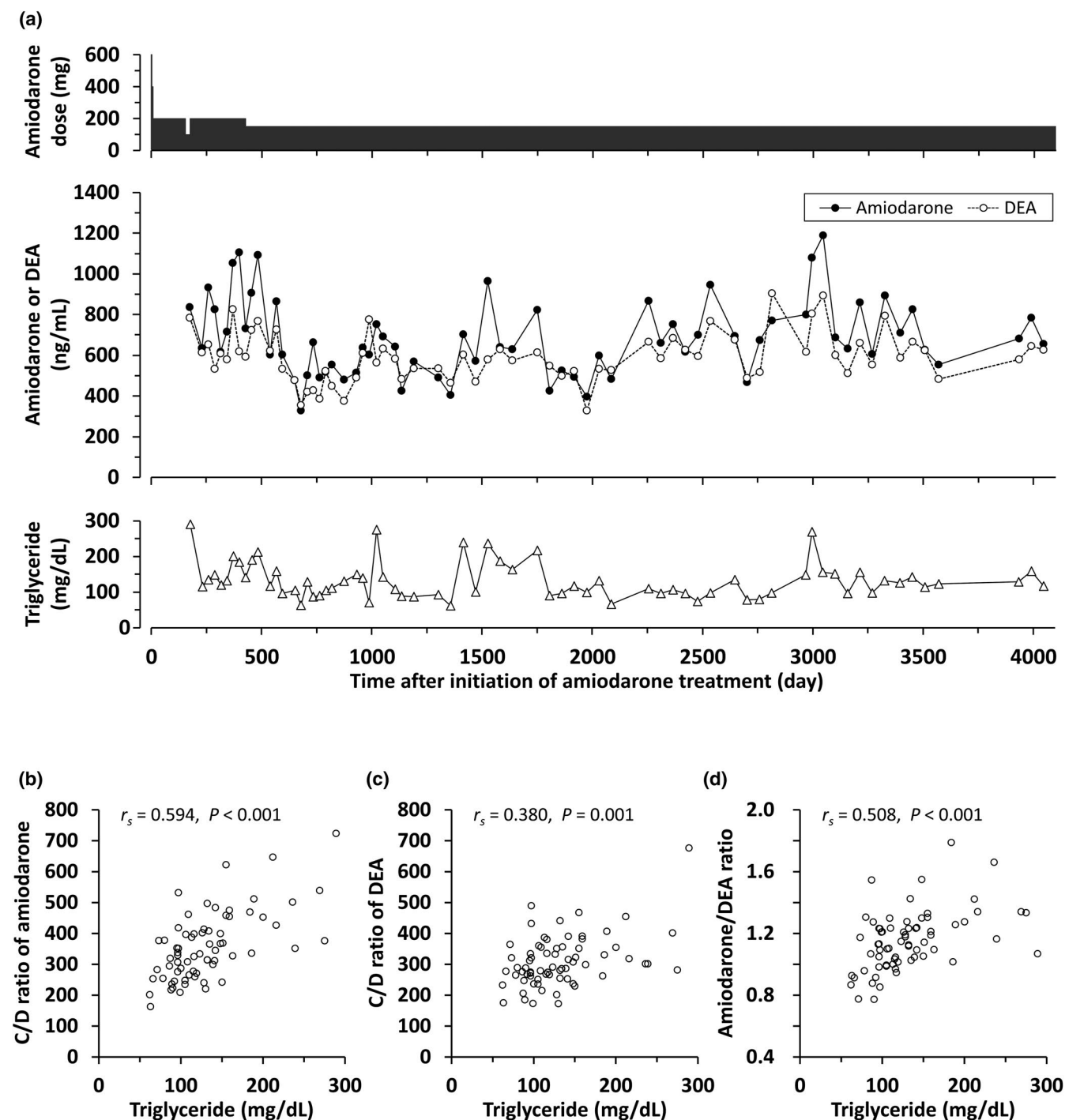

FIGURE 2 Concentration-to-dose (C/D) ratios of amiodarone (a, b) and desethylamiodarone (DEA; c, d), and the serum concentration ratio of amiodarone to DEA (amiodarone/DEA ratio; e, f) in the absence and presence of hypertriglyceridemia or hypercholesterolemia. Closed circles indicate outliers that are beyond the quartiles by one and a half interquartile range

158 ± 84 vs. 150 ± 144, $p = 0.008$). Among the patients who received co-administration of statins, the C/D ratios of amiodarone and DEA, and amiodarone/DEA ratio were higher in the hypertriglyceridemic state than those in the normotriglyceridemic state (amiodarone: 524 ± 205 vs. 384 ± 207, $p < 0.001$; DEA: 366 ± 133 vs. 333 ± 189, $p = 0.056$; amiodarone/DEA ratio: 1.46 ± 0.34 vs. 1.20 ± 0.36, $p < 0.001$).

Change of serum amiodarone concentrations in a typical case with hyperlipidemia

Serum concentrations of amiodarone and DEA as well as serum triglyceride levels were monitored in a male patient with atrial fibrillation, dilated cardiomyopathy, and hyperlipidemia (Figure 3a). In this patient, the fluctuation

FIGURE 3 Profiles of serum concentrations of amiodarone, desethylamiodarone (DEA), and triglyceride in a male patient receiving oral amiodarone for chronic atrial fibrillation (a). Influence of serum triglyceride levels on the concentration-to-dose (C/D) ratios of amiodarone (b) and DEA (c), and the serum concentration ratio of amiodarone to DEA (amiodarone/DEA ratio; d) within the patient

of serum amiodarone concentration corresponded with the change in serum triglyceride levels (Figure 3a). Additionally, the C/D ratios of amiodarone and DEA were significantly higher in the hypertriglyceridemic state than those in the normotriglyceridemic state (amiodarone: 460 ± 118 vs. 322 ± 81, $p < 0.001$; DEA: 363 ± 98 vs. 292 ± 68, $p = 0.003$; Figure 3b,c). The amiodarone/DEA ratio was also significantly higher in the hypertriglyceridemic state (1.28 ± 0.19 vs. 1.11 ± 0.17; $p = 0.002$). Furthermore, positive correlations were observed between the C/D ratio of amiodarone ($r_s = 0.594$; Figure 3b) or the amiodarone/DEA ratio ($r_s = 0.508$; Figure 3d) and serum triglyceride levels.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, which act as a biomarker for deterioration of heart failure and atrial fibrillation,[20] were markedly decreased from 6464 pg/ml at baseline to 110.6 ± 75.3 pg/ml after amiodarone administration. Moreover, under amiodarone treatment, the frequency at which NT-proBNP levels were greater than 125 pg/ml[21] tended to be reduced in the hypertriglyceridemic state relative to in the normotriglyceridemic state (12% vs. 30%; Figures S2).
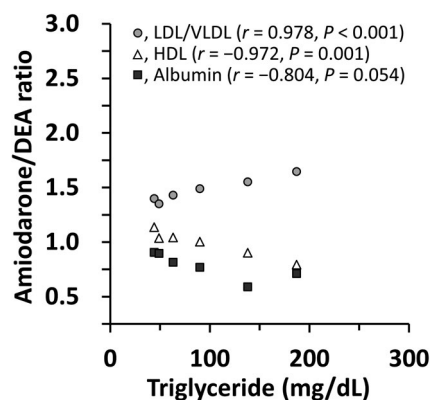
Distribution of amiodarone and DEA in serum lipoprotein and albumin fractions

Serum amiodarone distribution was investigated in lipoproteins and albumin fractions separated from 18 serum samples in 13 patients receiving amiodarone. These distributions were then compared with those for the drug-spiked serum samples.

In serum from patients receiving amiodarone, the drug recoveries from the LDL/VLDL, HDL, and albumin fractions were $66.0\% \pm 8.4\%$, $22.2\% \pm 6.4\%$, and $9.4\% \pm 1.9\%$ for amiodarone, and $50.7\% \pm 7.7\%$, $23.0\% \pm 6.4\%$, and $13.3\% \pm 2.6\%$ for DEA, respectively. Amiodarone recovery in the LDL/VLDL fraction was positively correlated with serum triglyceride levels ($r = 0.860$), whereas those in the HDL and albumin fractions showed negative correlations ($r = -0.550$ and $r = -0.635$, respectively; Figure 4a). Similar results were obtained for DEA recoveries (Figure 4b). Amiodarone/DEA ratios were positively correlated with serum triglyceride levels in the LDL/VLDL ($r = 0.781$), HDL ($r = 0.612$), and albumin fractions ($r = 0.706$; Figure 4c).

In drug-spiked serum samples, the amiodarone/DEA ratio was significantly higher in the LDL/VLDL fraction than the ratios in the HDL and albumin fractions (1.48 ± 0.10 vs. 0.99 ± 0.11 and 0.78 ± 0.11, respectively;

FIGURE 5 Influence of serum triglyceride levels on the ratio of amiodarone to desethylamiodarone (amiodarone/DEA ratio) in low-density lipoprotein/very-low-density lipoprotein (LDL/VLDL), high-density lipoprotein (HDL), and albumin fractions separated from serum samples spiked with amiodarone and DEA at 500 ng/ml
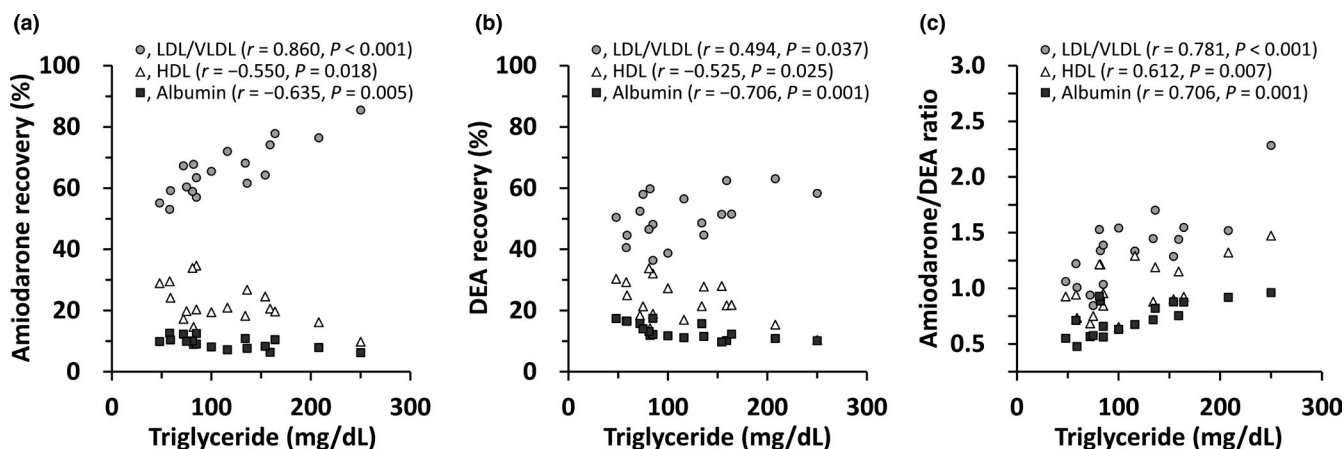
FIGURE 4 Influence of serum triglyceride levels on the distribution of amiodarone (a) and desethylamiodarone (DEA; b), and the ratio of amiodarone to DEA (amiodarone/DEA ratio; c) in low-density lipoprotein/very-low-density lipoprotein (LDL/VLDL), high-density lipoprotein (HDL), and albumin fractions separated from the serum of patients receiving oral amiodarone. The contents of amiodarone and DEA in each fraction are expressed as the fractional percentage recovery against the overall recovery from serum

$p < 0.05$; Figure 5). In the HDL and albumin fractions from drug-spiked serum, the correlation of the amiodarone/DEA ratio with serum triglyceride levels differed from those in the patient serum (Figure 4c), with a negative correlation observed for the HDL fraction ($r = -0.972$); furthermore, a negative correlation with no statistical significance was noted for the albumin fraction ($r = -0.804$, $p = 0.054$).

DISCUSSION

In the present study, we investigated the association of serum amiodarone concentration with serum lipid levels in patients. Our results show that serum amiodarone concentration increases in the hypertriglyceridemic state, which may be attributable to an increase in the circulating lipoprotein-bound form and decreased metabolism of amiodarone in this state. The increase in the lipoprotein-bound form of amiodarone arises from the high affinity of amiodarone to triglyceride-rich lipoproteins. This does not necessarily imply that the increase in total serum amiodarone concentration in the hypertriglyceridemic state leads to an increase in the unbound form of amiodarone, which is generally believed to be associated with pharmacological effects. These findings may account for the weak relationship between total serum concentration and the clinical efficacy/toxicity of amiodarone.

Serum concentration of amiodarone was associated with serum triglyceride levels in patients with hypertriglyceridemia (Figures 1 and 2), which led to an increased number of lipoprotein-bound forms in LDL/VLDL fractions. In contrast, serum amiodarone concentration was not correlated with total cholesterol. Serum amiodarone concentration increased in the hypertriglyceridemic state regardless of co-administration of statins, and sex and BMI, which were influencing factors for amiodarone clearance.[22] This phenomenon was also observed in the change in serum amiodarone concentrations for a typical case with normotriglyceridemic and hypertriglyceridemic states (Figure 3), in which concentrations were higher in the hypertriglyceridemic state. These findings are consistent with previous reports showing that plasma amiodarone concentration was considerably high in experimental hyperlipidemic rats.[16,23] Our present results from patient serum and drug-spiked serum also confirm that amiodarone is preferentially distributed in the LDL/VLDL fraction and that higher recoveries in the fraction are found with higher serum triglyceride levels (Figures 4 and 5). The altered distribution of amiodarone in the LDL/VLDL fraction may affect the proportion of the unbound form in the serum. The unbound form of amiodarone is reported to markedly decrease in hyperlipidemic rats due to a shift of amiodarone into the triglyceride-rich lipoprotein

fraction,[16] which leads to decreased amiodarone distribution into peripheral tissues.[17] The increase in total serum amiodarone concentration in hypertriglyceridemia may not reflect the increase in the unbound form responsible for the drug action.

We found that hepatic metabolism of amiodarone decreased in the hyperlipidemic state; hence, amiodarone/DEA ratios increased with the elevation of serum triglyceride levels in patients receiving amiodarone (Figures 1 and 4). These results were observed not only for total serum concentration but also for serum lipoproteins and albumin fractions separated from patient serum. Several studies have reported that hyperlipidemia impairs amiodarone metabolism in rats through decreased protein expression of the hepatic enzymes, CYP2C11 and CYP3A1/2, responsible for amiodarone metabolism in these animals.[17,24] In human subjects, hyperlipidemia decreases the CYP3A activity responsible for amiodarone metabolism[25]; therefore, impaired amiodarone metabolism in patients with hyperlipidemia may be attributable to reduced hepatic drug metabolizing activity. Because the intrinsic clearance of amiodarone is markedly reduced in cultured primary hepatocytes that are co-incubated with hyperlipidemic serum,[24] lipoprotein-bound amiodarone seems to hardly be metabolized to DEA (Figure 4c). The increased lipoprotein-bound form of amiodarone in triglyceride-rich lipoproteins may not participate in hepatic amiodarone metabolism, despite the previously reported expectation of LDLR-mediated hepatic uptake of amiodarone.[15]

Therapeutic drug monitoring of amiodarone is challenging because clear relationships do not exist between total serum concentration and clinical effects/toxicities of the drug.[5,6] Estimating the tissue distribution of amiodarone in hyperlipidemia could help provide an understanding of total serum concentration in amiodarone therapeutic drug monitoring. Previously, an experimental study reported that hyperlipidemia increases the electrophysiologic effect of amiodarone (QT prolongation) by increasing heart amiodarone concentrations.[23] The VLDL receptor, which is a member of the LDLR family expressed in fatty acid active tissues (e.g., the heart, muscle, and adipose tissue), plays a role in the uptake of triglyceride-rich lipoproteins in the heart[26]; therefore, it may contribute to the transport of amiodarone to the cardiomyocytes (i.e., the site of action for amiodarone). In the present study, a patient maintained lower NT-proBNP levels in the hypertriglyceridemic state during amiodarone therapy, indicating the potential for better control of atrial fibrillation. This result may also be associated with increased heart amiodarone concentrations via VLDL receptor-mediated tissue uptake in the hypertriglyceridemic state. Additionally, a clinical study reported that obesity, which frequently co-exists with hyperlipidemia, is a risk factor for

amiodarone-associated interstitial pneumonia in patients under long-term administration.[27] The present results and previous reports indicate the necessity to further investigate the association between the lipoprotein-binding of amiodarone and the clinical efficacy/toxicity of the drug in the hypertriglyceridemic state.

Our study has several limitations regarding data pertaining to serum amiodarone and DEA concentrations, and data collection. First, the unbound forms of amiodarone and DEA were not measured because the fraction unbound in plasma was considerably low (<0.001).[28] Second, NT-proBNP data were collected only in a typical case.

In conclusion, we found that serum amiodarone concentrations increased in the hypertriglyceridemic state through increased distribution of amiodarone in the LDL/VLDL fraction as well as decreased hepatic metabolism of amiodarone. The increased lipoprotein-binding of amiodarone in the hypertriglyceridemic state could potentially affect the delivery of amiodarone to target tissues and modify pharmacological effects. Overall, these findings provide new insights into the differences in pharmacokinetics and pharmacodynamics of amiodarone associated with normotriglyceridemic and hypertriglyceridemic states.

CONFLICT OF INTEREST

The authors declare no competing interests for this work.

AUTHOR CONTRIBUTIONS

N.H., K.D., and M.H. wrote the manuscript. K.D. and M.H. designed the research. N.H., K.D., S.K., K.A., M.I., and M.H. performed the research. N.H. and K.D. analyzed the data.

REFERENCES

1. Page RL, Joglar JA, Caldwell MA, et al. 2015 ACC/AHA/HRS guideline for the management of adult patients with supraventricular tachycardia: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Circulation*. 2016;133:e506-e574.
2. Al-Khatib SM, Stevenson WG, Ackerman MJ, et al. 2017 AHA/ACC/HRS guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Circulation*. 2018;138:e272-e391.
3. Stelfox HT, Ahmed SB, Fiskio J, Bates DW. Monitoring amiodarone's toxicities: recommendations, evidence, and clinical practice. *Clin Pharmacol Ther*. 2004;75:110-122.
4. Moore BM, Cordina RL, McGuire MA, Celermajer DS. Adverse effects of amiodarone therapy in adults with congenital heart disease. *Congenit Heart Dis*. 2018;13:944-951.
5. Aonuma K, Shiga T, Atarashi H, et al. Guidelines for therapeutic drug monitoring of cardiovascular drugs clinical use of blood drug concentration monitoring (JCS 2015) — digest version. *Circ J*. 2017;81:581-612.
6. Jürgens G, Graudal NA, Kampmann JP. Therapeutic drug monitoring of antiarrhythmic drugs. *Clin Pharmacokinet*. 2003;42:647-663.
7. Ohyama K, Nakajima M, Nakamura S, Shimada N, Yamazaki H, Yokoi T. A significant role of human cytochrome P450 2C8 in amiodarone N-deethylation: an approach to predict the contribution with relative activity factor. *Drug Metab Dispos*. 2000;28:1303-1310.
8. Holt DW, Tucker GT, Jackson PR, Storey GC. Amiodarone pharmacokinetics. *Am Heart J*. 1983;106:840-847.
9. Latini R, Tognoni G, Kates RE. Clinical pharmacokinetics of amiodarone. *Clin Pharmacokinet*. 1984;9:136-156.
10. Roden DM. Mechanisms underlying variability in response to drug therapy: implications for amiodarone use. *Am J Cardiol*. 1999;84:29R-36R.
11. Adams PC, Holt DW, Storey GC, Morley AR, Callaghan J, Campbell RW. Amiodarone and its desethyl metabolite: tissue distribution and morphologic changes during long-term therapy. *Circulation*. 1985;72:1064-1075.
12. Lalloz MR, Byfield PG, Greenwood RM, Himsworth RL. Binding of amiodarone by serum proteins and the effects of drugs, hormones and other interacting ligands. *J Pharm Pharmacol*. 1984;36:366-372.
13. Wasan KM, Brocks DR, Lee SD, Sachs-Barrable K, Thornton SJ. Impact of lipoproteins on the biological activity and disposition of hydrophobic drugs: implications for drug discovery. *Nat Rev Drug Discov*. 2008;7:84-99.
14. Shayeganpour A, Lee SD, Wasan KM, Brocks DR. The influence of hyperlipoproteinemia on in vitro distribution of amiodarone and desethylamiodarone in human and rat plasma. *Pharm Res*. 2007;24:672-678.
15. Yamamoto H, Takada T, Yamanashi Y, et al. VLDL/LDL acts as a drug carrier and regulates the transport and metabolism of drugs in the body. *Sci Rep*. 2017;7:633.
16. Shayeganpour A, Jun AS, Brocks DR. Pharmacokinetics of amiodarone in hyperlipidemic and simulated high fat-meal rat models. *Biopharm Drug Dispos*. 2005;26:249-257.
17. Shayeganpour A, Korashy H, Patel JP, El-Kadi AOS, Brocks DR. The impact of experimental hyperlipidemia on the distribution and metabolism of amiodarone in rat. *Int J Pharm*. 2008;361:78-86.
18. Pollak PT, Bouillon T, Shafer SL. Population pharmacokinetics of long-term oral amiodarone therapy. *Clin Pharmacol Ther*. 2000;67:642-652.
19. Hata Y, Mabuchi H, Saito Y, et al. Report of the Japan Atherosclerosis Society (JAS) guideline for diagnosis and treatment of hyperlipidemia in Japanese adults. *J Atheroscler Thromb*. 2002;9:1-27.
20. Patton KK, Ellinor PT, Heckbert SR, et al. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the cardiovascular health study. *Circulation*. 2009;120:1768-1774.
21. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129-2200.

22. Araki R, Yukawa E, Nakashima MN, et al. Population pharmacokinetic investigation for optimization of amiodarone therapy in Japanese patients. *Ther Drug Monit*. 2011;33: 750-756.

23. Hamdy DA, Brocks DR. Experimental hyperlipidemia causes an increase in the electrocardiographic changes associated with amiodarone. *J Cardiovasc Pharmacol*. 2009;53:1-8.

24. Brocks DR, Hamdy DA, Ben-Eltriki M, Patel JP, El-Kadi AO. Effect of rat serum lipoproteins on mRNA levels and amiodarone metabolism by cultured primary rat hepatocytes. *J Pharm Sci*. 2013;102:262-270.

25. Willrich MAV, Rodrigues AC, Cerda A, et al. Effects of atorvastatin on CYP3A4 and CYP3A5 mRNA expression in mononuclear cells and CYP3A activity in hypercholeresterolemic patients. *Clin Chim Acta*. 2013;421:157-163.

26. Chung NS, Wasan KM. Potential role of the low-density lipoprotein receptor family as mediators of cellular drug uptake. *Adv Drug Deliv Rev*. 2004;56:1315-1334.

27. Koike H, Fujino T, Koike M, et al. Obesity is associated with the development of interstitial pneumonia under long-term administration of amiodarone in refractory atrial fibrillation patients. *Int Heart J*. 2016;57:30-34.

28. Chen Y, Mao J, Hop CE. Physiologically based pharmacokinetic modeling to predict drug-drug interactions involving inhibitory metabolite: a case study of amiodarone. *Drug Metab Dispos*. 2015;43:182-189.

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