Amiodarone disrupts cholesterol biosynthesis pathway and causes accumulation of circulating desmosterol by inhibiting 24-dehydrocholesterol reductase

■ P. Simonen[1], S. Li[2,3], N. K. Chua[4], A.-M. Lampi[5], V. Piironen[5], J. Lommi[1], J. Sinisalo[1], A. J. Brown[4] (iD), E. Ikonen[2,3,†] & H. Gylling[6,†] (iD)

From the [1]Heart and Lung Center, Cardiology, University of Helsinki and Helsinki University Hospital, Helsinki; [2]Department of Anatomy and Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Helsinki; [3]Minerva Foundation Institute for Medical Research, Helsinki, Finland; [4]School of Biotechnology and Biomolecular Sciences, UNSW Sydney, Sydney, NSW, Australia; [5]Department of Food and Nutrition, University of Helsinki, Helsinki; and [6]University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Abstract. Simonen P, Li S, Chua NK, Lampi A-M, Piironen V, Lommi J, Sinisalo J, Brown AJ, Ikonen E, Gylling H (University of Helsinki and Helsinki University Hospital; University of Helsinki; Minerva Foundation Institute for Medical Research, Helsinki, Finland; UNSW Sydney, Sydney, NSW, Australia; University of Helsinki, Helsinki, Finland). Amiodarone disrupts cholesterol biosynthesis pathway and causes accumulation of circulating desmosterol by inhibiting 24-dehydrocholesterol reductase. *J Intern Med* 2020; **288**: 560–569.

Background. We have earlier reported that amiodarone, a potent and commonly used antiarrhythmic drug increases serum desmosterol, the last precursor of cholesterol, in 20 cardiac patients by an unknown mechanism.

Objective. Here, we extended our study to a large number of cardiac patients of heterogeneous diagnoses, evaluated the effects of combining amiodarone and statins (inhibitors of cholesterol synthesis at the rate-limiting step of hydroxymethyl-glutaryl CoA reductase) on desmosterol levels and investigated the mechanism(s) by which amiodarone interferes with the metabolism of desmosterol using *in vitro* studies.

Methods and Results. We report in a clinical case–control setting of 236 cardiac patients (126 with and 110 without amiodarone treatment) that amiodarone medication is accompanied by a robust increase in serum desmosterol levels independently of gender, age, body mass index, cardiac and other diseases, and the use of statins. Lipid analyses in patient samples taken before and after initiation of amiodarone therapy showed a systematic increase of desmosterol upon drug administration, strongly arguing for a direct causal link between amiodarone and desmosterol accumulation. Mechanistically, we found that amiodarone resulted in desmosterol accumulation in cultured human cells and that the compound directly inhibited the 24-dehydrocholesterol reductase (DHCR24) enzyme activity.

Conclusion. These novel findings demonstrate that amiodarone blocks the cholesterol synthesis pathway by inhibiting DHCR24, causing a robust accumulation of cellular desmosterol in cells and in the sera of amiodarone-treated patients. It is conceivable that the antiarrhythmic potential and side effects of amiodarone may in part result from inhibition of the cholesterol synthesis pathway.

Keywords: amiodarone, desmosterol, DHCR24, cholesterol biosynthesis, cholesterol absorption.

Introduction

Amiodarone has been used clinically for more than half a century and is the most commonly used potent antiarrhythmic drug to suppress atrial and ventricular tachyarrhythmias [1]. It acts through multiple mechanisms, including prolongation of repolarization, reduction of excitability and slowing of conduction [2]. It is an iodinated derivative of benzofuran and is metabolized in the liver to an active metabolite desethylamiodarone (DEA). Amiodarone has a very long half-life of approximately 60 days and has several adverse effects, adding up to 15% during the first year of treatment

†Contributed equally.

and up to 50% during long-term use [1]. The most serious adverse effects are pulmonary and hepatic toxicity and abnormalities in thyroid function. The benzofuran ring is highly lipophilic, resulting in effective distribution of the drug into different organs and cells, where the drug is assumed to cause structural and functional disturbances, a 'lipid traffic jam' [3,4]. It is not clear whether the amiodarone-induced lipid accumulation induces the organ toxicities described during amiodarone treatment.

We have earlier described in a limited number of cardiac patients with myocardial inflammatory disease (i.e. 20 patients with cardiac sarcoidosis or giant cell myocarditis) that amiodarone affected cholesterol biosynthesis [5]. An unexpected finding in amiodarone-treated patients was that the concentration of serum desmosterol, the last precursor of cholesterol in the Bloch unsaturated sterol side chain pathway, was increased on average 12-fold compared with the control subjects [5]. The mechanism by which amiodarone increases circulating desmosterol is not known [6]. However, desmosterol can regulate inflammatory responses [7], raising the possibility that the observed desmosterol accumulation upon amiodarone treatment in this study was somehow related to inflammatory cardiac diseases.

The aim of the present study was to evaluate in a clinical case–control setting if the amiodarone-induced increase in serum desmosterol levels can be reproduced in a large and heterogeneous group of cardiac patients excluding inflammatory myocardial disease, and whether it depends on gender, age, body mass index (BMI) or different diseases. In addition, we evaluated whether the inhibition of hydroxy-methyl-glutaryl CoA reductase (HMGCR) by statins interfered with the amiodarone-induced effects on cholesterol precursors and whole-body cholesterol metabolism. Finally, we investigated the detailed mechanism(s) by which amiodarone interfered with the metabolism of desmosterol using *in vitro* studies.

Materials and methods

Clinical study population

The study population consisted of 236 consecutive cardiac patients, 174 men and 62 women with a mean age of 61 ± 1 (SE) years, who were referred to the Department of Cardiology, Helsinki University Hospital in the years 2018–2019 because of various cardiac problems. Of these patients, we selected 126 consecutive subjects who were on long-term amiodarone treatment for severe arrhythmias (amiodarone group), and 110 age- and sex-matched controls without amiodarone treatment (control group). The primary cardiac diagnoses in the control group were matched as much as possible with those of the amiodarone group. Amiodarone and lipid-lowering treatment had to be unchanged for at least eight weeks before the study. In-hospital patients were studied when they had reached a stable state of health. All subjects gave their written informed consent. The study was performed according to the principles of the Declaration of Helsinki. The Ethics Committee for the Department of Medicine, Hospital District of Helsinki and Uusimaa approved the study protocol.

Clinical study design

Blood samples were drawn after a 12-h fasting. The subjects were weighed and their height was measured, BMI was calculated as weight (kg)/height (m)^2, and clinical data including medical history and symptoms, laboratory tests (especially alanine aminotransferase and total or free T4), and current drug treatments were recorded. The in-hospital subjects were studied on the standard hospital diet and outpatients on their normal habitual diet.

Clinical study methods

Serum cholesterol and noncholesterol sterols zymostenol (called cholestenol in our previous study) [5], desmosterol, lathosterol and squalene (i.e. cholesterol precursors), and campesterol, sitosterol, stigmasterol and avenasterol (i.e. plant sterols), and cholestanol (5α-saturated derivative of cholesterol) were analysed using gas–liquid chromatography (GLC) with a 50-m capillary column (Ultra 2, Agilent Technologies, Wilmington, DE) and flame ionization detection with 5α-cholestane as internal standard [8]. The identities of the sterol peaks were further verified by mass spectrometry [5]. Serum concentrations of the noncholesterol sterols and squalene were adjusted to cholesterol in the same GLC run and expressed as ratios to cholesterol (10^2 μmol mmol^{-1} cholesterol) in order to enable their comparison between subjects and populations with different serum cholesterol levels. Therefore, serum noncholesterol sterols are given

as ratios to cholesterol herein. Serum triglyceride concentrations were analysed enzymatically using an automated analyser (Thermo Fisher Scientific Indiko™).

Sterol measurements in A431 cells

A431 cells (ATCC, cat. no. CRL-1555) were cultured in DMEM (Lonza), supplemented with 10% (v/v) FBS, penicillin/streptomycin (100 U mL^{-1} each), L-glutamine (2 mmol L^{-1}) at 37°C in 5% CO_2. Mycoplasma testing was performed regularly using PCR detection. The 24-dehydrocholesterol reductase (DHCR24) knockout (KO) A431 cell line was generated with CRISPR/Cas9 technology. Briefly, cells were transfected with Cas9 nickase plus a pair of sgRNAs (target sites: DHCR24-1A, AGGGCGCCCGTGCACATGA AGG and DHCR24-1B: TCACTGTCTCACTACGTGTC GGG) [9]. Single clones were isolated as described [10], and clones lacking DHCR24 were identified by Western blotting using an anti-DHCR24 antibody (Santa Cruz, D-10, 1:500). For sterol measurements, cells were delipidated by culturing in serum-free medium supplemented with 5% lipoprotein-deficient serum for 3 days and treated with amiodarone (Cayman, 15213), N-desethylamiodarone DEA (Cayman, 9000537) or solvent (DMSO) only for the final 48 h. Cells were washed, scraped into ice-cold PBS and pelleted. Protein concentrations were measured using the Bio-Rad DC assay. Lipids were extracted with chloroform–methanol, saponified with potassium hydroxide in ethanol, extracted with hexane and silylated with trichloromethylsilane. Sterols (cholesterol, lanosterol, zymosterol, zymostenol and desmosterol) were separated and quantified by capillary GLC as described above.

DHCR24 activity assay

CHO-DHCR24-V5 cells were cultured in DMEM/F12 media, supplemented with 5% (v/v) lipoprotein-deficient serum, 150 μg mL^{-1} hygromycin B [11]. Cells were plated and grown for 24 h before treating with DMSO, amiodarone (Sigma-Aldrich, A8423) or triparanol (Cayman, 20918) for 48 h. During the final 4 h of the treatment, cells were treated with [^{2}H$_6$]-desmosterol complexed to methyl-β-cyclodextrin, followed by lipid extraction and derivatization with N,O-Bis(trimethylsilyl)trifluoroacetamide for GC-MS analyses. The m/z values for the ions detected in selective ion monitoring mode are as previously detailed (11): 5α-cholestane: m/z = 217.30, 372.4, D6-cholesterol:

m/z = 374.41, 464.6, D6-desmosterol: m/z = 333.24, 462.43.

To assay for DHCR24 enzyme activity *in vitro*, we isolated crude microsomes from CHO-DHCR24-V5 cells. CHO-DHCR24-V5 cells were plated and grown in 150 mm dish for 24 h. Next, crude microsomal membranes were isolated as previously described [12] and protein content was quantified using BCA assay (Thermo Fisher Scientific). The assay contained 15 μg microsomal protein, 100 mmol L^{-1} Tris-HCl (pH 7.23), 0.1 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol, 30 mmol L^{-1} nicotinamide, 5 mmol L^{-1} NADPH, 20 μmol L^{-1} FAD, 30 mmol L^{-1} glucose-6-phosphate, 500 μg mL^{-1} bovine serum albumin, 2 U glucose-6-phosphate dehydrogenase (for NADPH regeneration), 1 μg mL^{-1} [^{2}H$_6$]-desmosterol (Avanti, 700040P) complexed to methyl-β-cyclodextrin and the indicated treatments (either DMSO, amiodarone or triparanol) in a 50 μL volume. All components except substrate [^{2}H$_6$]-desmosterol were prepared whilst on ice and pre-incubated in a 37°C water bath for 10 min to allow binding of inhibitors to DHCR24. Next, the [^{2}H$_6$]-desmosterol was added and the reaction continued for 2 h in the 37°C water bath. The reaction was terminated by adding 500 μL 0.1 mol L^{-1} NaOH. Lipids were then extracted and derivatized for GC-MS analyses as described previously [13]. Quantification of DHCR24 activity was based on chromatographic peak areas measured for the product, [^{2}H$_6$]-cholesterol, relative to the precursor, [^{2}H$_6$]-desmosterol.

Statistical analyses

The statistical analyses were performed with SPSS for Windows 22.0 (SPSS, Chicago, IL). Sample size calculation was based on significance levels (α = 0.05 and β = 0.20) and essential information provided from a previous study [5]. Using these estimates, the size of the required population was appropriate. Normality and homogeneity of variance assumptions were checked before further analyses, and variables not normally distributed were transformed logarithmically. Continuous variables in the groups were compared by analysis of variance, and the noncontinuous variables were tested by using the chi-square or Fisher's exact test. Variables not normally distributed even after logarithmic transformation or nonhomogenous in variance were tested with Kruskal–Wallis and Mann–Whitney

U-tests. Spearman's correlation coefficients were calculated. A *P*-value of <0.05 was considered statistically significant, whereas a *P*-value of >0.05 was denoted as nonsignificant (NS). The results are expressed as mean ± SE.

Results

Clinical study

Study population and clinical characteristics
In the amiodarone (*n* = 126) and control (*n* = 110) groups, age and BMI were similar, and 74% of the subjects were men in both groups (Table 1). Age and BMI were also similar between gender in the groups. In the amiodarone group, ventricular tachyarrhythmias were more frequent than in the control group (*P* < 0.05), whereas atrial tachyarrhythmias were similar between the groups. The number of cases with diagnosis of cardiomyopathy (CMP), either hypertrophic or dilated, or coronary artery disease (CAD) was comparable

between the study groups as were congenital anomalies or valvular defects (9% in the amiodarone vs 5% in the control group (NS)) and the frequency of type 2 diabetes (13% in the amiodarone and 11% in the control group (NS)). In the amiodarone and control groups, 48% and 42% of the subjects were on statin treatment (NS), and in both groups, 80% of the subjects on statin were males. In both groups, one subject was on ezetimibe treatment and two subjects had a combination therapy of statin + ezetimibe. In the control group, one and in the amiodarone group two subjects were on low-dose corticosteroid therapy. In the amiodarone group, the median daily dose of amiodarone was 200 mg with a range of 100–400 mg. No signs of thyroid, pulmonary or liver toxicity were observed in the patients at the time of our study.

Serum lipids
We measured cholesterol, noncholesterol sterols and triglycerides in the patients' sera. The ratios to

Table 1. *Demographics, main clinical characteristics, serum cholesterol, triglycerides and noncholesterol sterols in the study population*

Variable	Control group (*n* = 110)	Amiodarone group (*n* = 126)
Gender, M/F, *n*	81/29	93/33
Age, years	60.5 ± 1.0	61.9 ± 0.9
Body mass index, kg/m^2	28.0 ± 0.4	28.0 ± 0.4
Ventricular tachyarrhythmias, %	12	25*
Atrial tachyarrhythmias, %	68	75
CMP or CAD, %	25	37
Statin treatment, yes/no, *n*	46/64	61/65
Serum cholesterol, mmol/L	4.58 (2.10–9.40)	4.41 (1.54–7.98)
Serum triglycerides, mmol/L	1.30 (0.44–6.11)	1.16 (0.51–6.47)
Cholesterol precursors		
Squalene[a]	13 (4–122)	12 (3–51)
Zymostenol[a]	16 (4–38)	37 (10–182)*
Desmosterol[a]	94 (37–164)	696 (171–3107)*
Lathosterol[a]	103 (9–291)	50 (7–266)*
Plant sterols and cholestanol		
Campesterol[a]	217 (61–827)	269 (99–986)
Stigmasterol[a]	10 (5–29)	12 (5–34)*
Sitosterol[a]	124 (35–485)	162 (59–626)
Avenasterol[a]	38 (17–106)	45 (23–146)
Cholestanol[a]	146 (60–266)	181 (60–367)*

Mean ± SE, median (range), *n*, %. In the analysis of the lipids and sterols, statin treatment was used as a covariate.
CAD, coronary artery disease; CMP, cardiomyopathy.
[a]10^{2}µmol/mmol cholesterol.
*Significantly different from control subjects.

cholesterol of serum cholesterol precursors are markers of cholesterol synthesis, whilst plant sterols and cholestanol are markers of cholesterol absorption efficiency [14,15]. Statin treatment was taken as a covariate in all serum lipid analyses. The concentrations of serum total cholesterol and triglycerides did not differ significantly between the study groups (Table 1), reversing our previous finding of elevated serum triglyceride concentrations by amiodarone in a pilot study (5). Neither gender nor the low-dose corticosteroid therapy had any effect on serum cholesterol, triglycerides or noncholesterol sterols in the groups.

Of the serum cholesterol precursors, desmosterol and zymostenol were ~7- and ~2-fold higher and lathosterol 50% lower, on average, in the amiodarone than in the control group ($P < 0.001$ for all; Table 1). Serum desmosterol correlated with the daily amiodarone dose ($r = 0.365$, $P < 0.001$), but was independent of age, gender, BMI, lipid-lowering treatment or the type of cardiac disease in the amiodarone-treated patients.

The moderate increase in serum zymostenol, already observed in our previous study (5), indicates that amiodarone also interfered with the Kandutsch–Russell saturated sterol side chain pathway. This can be explained by the *in vitro* observation that amiodarone inhibits the activity of

$\Delta 8$–$\Delta 7$ isomerase (also known as EBP), blocking the conversion of zymostenol to lathosterol[16] and, in fact, serum lathosterol was lowered by amiodarone (Table 1). Of the cholesterol absorption markers, serum stigmasterol and cholestanol were elevated in the amiodarone compared with the control group. In spite of these interferences, serum cholesterol precursors correlated significantly inversely with serum absorption markers in the amiodarone and control groups (e.g. lathosterol vs. campesterol $r = -0.455$, $P < 0.001$ in the amiodarone and $r = -0.436$, $P < 0.001$ in the control group), as well as in both groups without and with statin treatment, suggesting intact cholesterol homeostatic regulation in all groups.

Statin treatment affected serum cholesterol and the noncholesterol sterols alike in the amiodarone and the control groups. Thus, serum cholesterol and the cholesterol precursors were lower and the plant sterols and cholestanol higher in statin + than statin- subjects in both groups, as shown for serum cholesterol, desmosterol and campesterol in Fig. 1. In fact, the plant sterols and cholestanol were even higher in the statin + amiodarone than statin + control group, as shown for campesterol in Fig. 1.

In the amiodarone group, we had access to serum samples taken before and during amiodarone
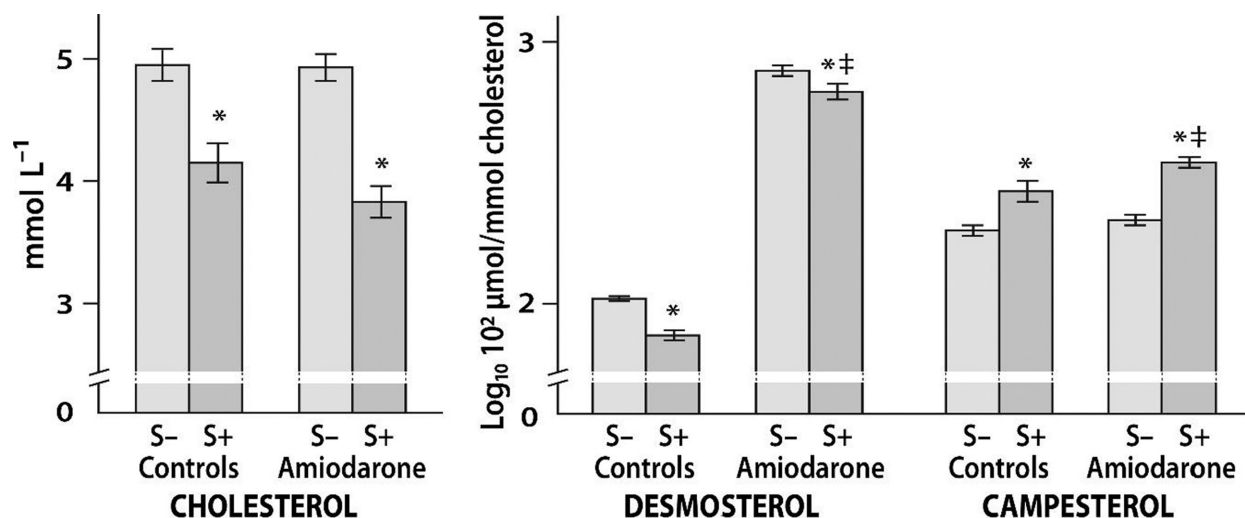
Fig. 1 *Serum cholesterol concentration and the ratios to cholesterol of desmosterol and campesterol in subjects without (S−) and with (S+) statin treatment in the control (n = 110, S − n = 64, S + n = 46) and amiodarone (n = 126, S − n = 65, S + n = 61) groups. Mean ± SE. *significantly different from S − subjects, ‡significantly different from S + control subjects.*
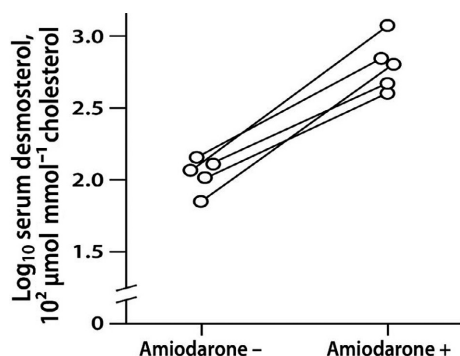
Fig. 2 *Serum desmosterol to cholesterol ratio before (Amiodarone −) and with amiodarone treatment (Amiodarone +) in five subjects in the amiodarone group.*

treatment in five subjects. In each case, serum desmosterol levels were elevated during amiodarone treatment, with the increase of desmosterol ranging from 297 to 1071 10^2 μmol mmol^{-1} cholesterol (Fig. 2).

Effect of amiodarone on sterol levels in human cells

To analyse if amiodarone or its major metabolite *in vivo* DEA affects cellular sterol synthesis, we cultivated human A431 cells in lipoprotein-deficient serum (to increase endogenous cellular cholesterol synthesis) in the presence of amiodarone or DEA. For comparison, we generated A431 cells from which the enzyme converting desmosterol to cholesterol, DHCR24, was knocked out using CRISPR/Cas9 gene editing (DHCR24-KO cells). We found that amiodarone and DEA effectively increased cellular desmosterol levels and concomitantly reduced cholesterol levels (Fig. 3), suggesting that both compounds inhibit DHCR24 in cells. In addition, zymosterol and zymostenol accumulated in amiodarone or DEA treated cells. DHCR24-KO cells exhibited a pronounced accumulation of desmosterol but not zymosterol or zymostenol (Fig. 3), indicating that accumulation of the latter two sterols is not secondary to DHCR24 inhibition. Rather, amiodarone and DEA also partially blocked EBP (Δ8–Δ7 isomerase) activity, in agreement with previous findings [16].

*Effect of amiodarone on DHCR24 enzyme activity*

To test if amiodarone directly inhibits DHCR24, we set up an *in vitro* assay. As a proof-of-principle, we first tested amiodarone versus an established inhibitor of DHCR24, triparanol, in cell culture

using CHO-7 stable cells that express human DHCR24 (CHO-DHCR24-V5) [11]. Cells cultivated in lipoprotein-deficient serum were incubated with deuterated desmosterol for 4 h. We found that both amiodarone and triparanol inhibited DHCR24 activity in cell culture (Fig. 4a and b), demonstrated by the higher ratio of [^{2}H$_6$]-desmosterol to [^{2}H$_6$]-cholesterol in triparanol and amiodarone-treated cells (Fig. 4a, *bottom panels*), compared to DMSO control (Fig. 4a, *top right*). Unlabelled cells were included to distinguish peaks from cellular endogenous cholesterol and desmosterol from their deuterated counterparts (Fig. 4a, *top left*). To test if amiodarone directly inhibits DHCR24, we isolated microsomes from these cells and performed an *in vitro* enzyme activity assay. This demonstrated that amiodarone directly inhibits the activity of DHCR24 (Fig. 4c) across two concentrations.

Discussion

This study shows that in cardiac patients amiodarone increased the levels of the circulating cholesterol precursors, desmosterol and zymostenol ~7- and ~2-fold and decreased serum lathosterol level to a half, on average, irrespective of gender, age, BMI, or primary cardiac or other diseases, or the use of lipid-lowering drugs. We have previously demonstrated that amiodarone markedly elevated serum levels of desmosterol and to a lesser extent zymostenol in a limited number of subjects with inflammatory cardiac disease [5]. These findings were confirmed and expanded in the present larger, controlled and well-matched study. Amiodarone seemed to interfere with both Bloch and Kandutsch–Russell cholesterol synthesis pathways. Regarding the basic mechanisms, we revealed that amiodarone results in desmosterol accumulation in cultured human cells and that the compound directly inhibits the DHCR24 enzyme activity in microsomal membranes. Moreover, the increase in zymostenol was not secondary to DHCR24 inhibition, but rather amiodarone and DEA appeared to partially block Δ8–Δ7 isomerase activity, in keeping with previous findings [16].

Paradoxically, elevated serum desmosterol and zymostenol levels reflect upregulated whilst lowered serum lathosterol reflects downregulated whole-body cholesterol synthesis, as verified in different populations both at baseline and during lipid-lowering drug treatments [14,15,17]. It is possible that blocking the conversion of zymostenol to lathosterol by amiodarone resulted in low

(a)

(b)
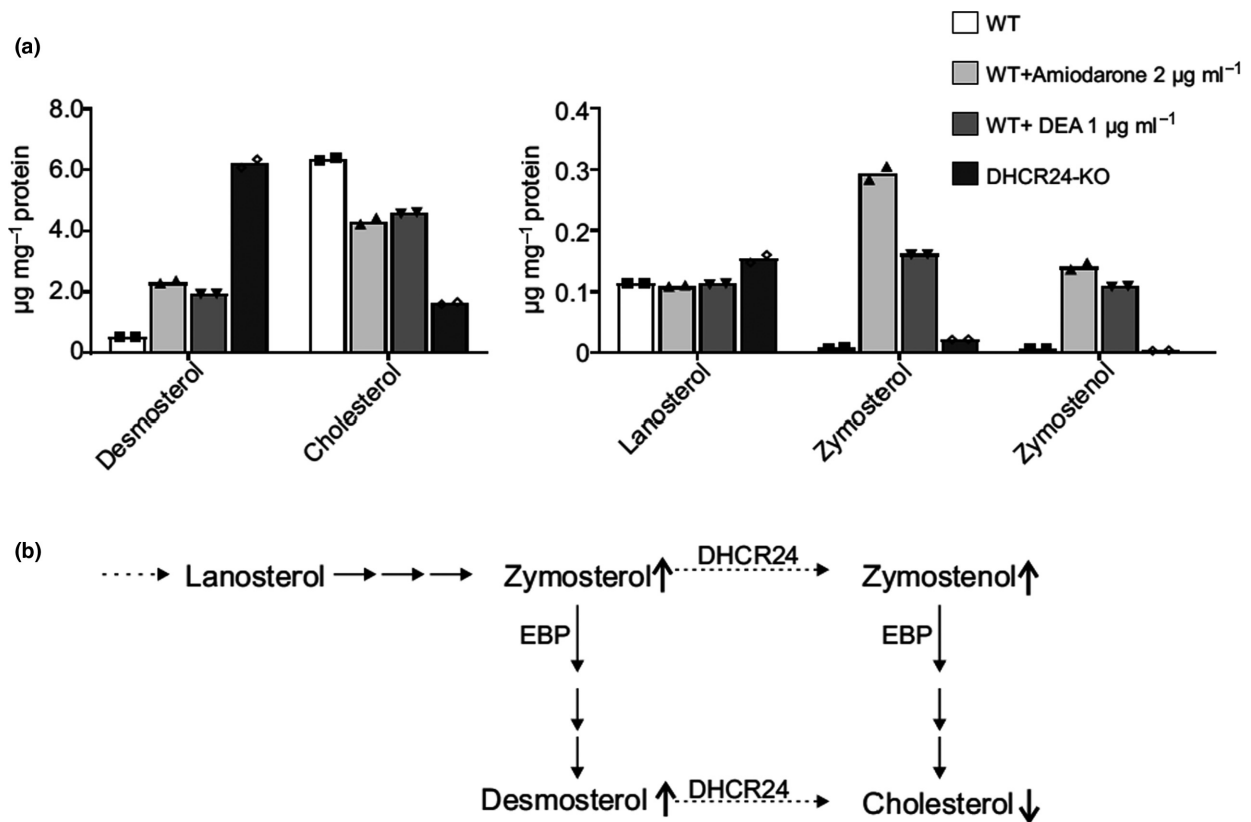
Fig. 3 *Effects of amiodarone and DEA on cellular sterol profile. (a) Sterol profiles of A431 cells treated with the indicated drug (WT = wild-type A431 cells treated with solvent only) for the final 48 h of the 3-day LPDS starvation. Two independent measurements for each. (b) Schematic cartoon of the findings in A with respect to the cholesterol synthesis pathway.*

lathosterol levels *in vivo*. Another possibility is that high desmosterol *per se* interfered with cholesterol homeostasis. In fact, desmosterol is a ligand for liver X receptor (LXR), and inhibits the activation of sterol response element-binding protein-2 (SREBP-2), thereby downregulating cholesterol synthesis [18,19]. However, the effect of amiodarone on LXR and SREBP-2 would require formal investigation.

Due to the increase of some but decrease of other cholesterol synthesis markers, it was not straight forward to determine the effect of amiodarone on whole-body cholesterol synthesis. However, elevated stigmasterol and cholestanol levels in the amiodarone group suggested increased cholesterol absorption efficiency. According to the reciprocal regulation of cholesterol synthesis and absorption [20], which, importantly, was intact in the amiodarone group, amiodarone most likely upregulated intestinal cholesterol absorption whilst

downregulating cholesterol synthesis. The tendency to steady state in human cholesterol metabolism explains the similar cholesterol concentrations in the amiodarone and control groups, unlike in the *in vitro* studies, where cellular cholesterol was decreased by amiodarone.

Regarding the combination of amiodarone with statins, several interesting observations were made. First, in the amiodarone group, the statin-induced inhibition of HMGCR was clearly only partial, since we still observed the desmosterol accumulation in this group. Metabolic studies have demonstrated that the statin-induced reduction in whole-body cholesterol synthesis is ~40% [21], but this may vary between statins and dosage. Second, a more significant upregulation of the absorption markers with the combination of statin + amiodarone treatment compared to statin alone may result from the fact that amiodarone increases the circulating
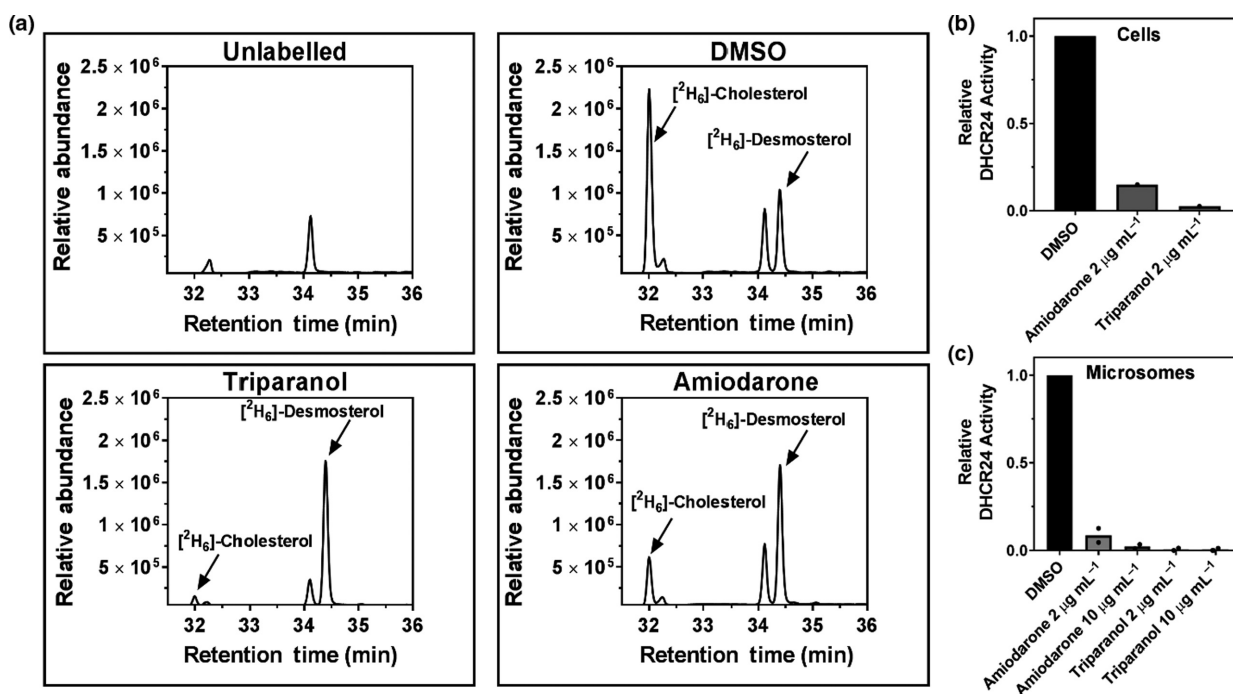
Fig. 4 *Amiodarone and triparanol inhibit DHCR24 enzyme activity. (a) Chromatograms from a cell culture experiment as proof-of-principle for DHCR24 inhibition. (b) CHO-DHCR24-V5 cells were treated with the indicated inhibitors for 48 h. DMSO concentration was kept at 0.2% (v/v) in all conditions. Cells were labelled with $[^2H_6]$-desmosterol for 4 h and harvested for lipid extraction to analyse $[^2H_6]$-cholesterol levels relative to $[^2H_6]$-desmosterol levels. The $[^2H_6]$-cholesterol to $[^2H_6]$-desmosterol ratios were normalized so that the control condition was set to 1. (c) Microsomes from CHO-DHCR24-V5 cells were pretreated with the indicated inhibitors in an activity assay buffer in vitro for 10 min. Microsomes were then labelled with $[^2H_6]$-desmosterol for 2 h and harvested for lipid extraction for GC-MS analyses as in b. Data are mean from two independent experiments.*

concentration of statins by inhibiting CYP3A4; consequently, cholesterol absorption increases [17]. In addition, it is possible that blocking cholesterol synthesis at two steps (early and late) might give an enhanced compensatory increase in cholesterol and sterol absorption in general, and this may contribute to the similar cholesterol reduction with both synthesis inhibitors vs. statin alone. During amiodarone + statin treatment, inhibiting cholesterol absorption with phytostanols or ezetimibe could still lower serum cholesterol concentration. In fact, some patients in the amiodarone group had amiodarone + statin + ezetimibe combination therapy, with serum cholesterol concentrations within the goal values and without side effects (data not shown).

As previously proposed [5], the simplest explanation for the increase in serum desmosterol observed in response to amiodarone treatment is that this drug inhibits DHCR24, the enzyme reducing the double bond in the side chain of desmosterol to make cholesterol. Supporting this idea, we observed in cell experiments that amiodarone increased desmosterol at the expense of cholesterol (Figs 3, 4a and b). Moreover, we found using an isolated microsome system that amiodarone directly inhibits DHCR24 activity to a comparable extent as the archetypal DHCR24 inhibitor, triparanol (Fig. 4c). Amiodarone and triparanol share structural similarities [6]. In particular, they both possess a diethylaminoethoxy side chain which corresponds in length and general conformation to the sterol side chain [22]. It has been proposed that the lone electron of the nitrogen atom, which is situated at a position analogous to C-24 of the sterol, plays the same binding role as the Δ24 double bond electrons of the desmosterol substrate [23]. Further work would be required to determine if amiodarone and triparanol share the same

mechanism of inhibition, which for triparanol has been reported to be noncompetitive [24].

At therapeutic concentrations, amiodarone alters the functions of multiple ion channels controlling the cardiac action potential [25]. Importantly, these are integral membrane proteins that are affected by the surrounding lipid bilayer. Based on our results, amiodarone modifies the sterol composition of cell membranes, and it seems plausible that this may contribute to the multi-target effects of the drug. On the other hand, a major limitation of long-term amiodarone therapy is its broad and unusual side effect profile. The present data also open the possibility that some of the adverse effects might in fact result from DHCR24 inhibition. For instance, DHCR4 inhibition can lead to cataracts [26], a known side effect of amiodarone. However, the amiodarone-related transient cataract is less severe compared with that caused by triparanol treatment. The latter develops rapidly within 3 to 19 months from starting the therapy and leading to severe cataracts requiring operative treatment even in middle-aged subjects without any predisposing risk factors [27,28]. Also, the sensitivity of the thyroid to amiodarone-induced toxicity (hypo- or hyperthyroidism) might be linked to high levels of DHCR24 in the thyroid: DHCR24 is upregulated by thyroid hormones and thyroid hormone receptor [29,30]. Pulmonary side effects might, instead, be related to the phospholipidosis typically induced by cationic amphiphilic drugs, rather than direct enzyme inhibition. Regarding the liver toxicity, amiodarone can cause nonalcoholic steatohepatitis [31] and interestingly, preliminary results demonstrate that liver desmosterol accumulation was correlated with the severity of inflammation and steatosis [32].

Conclusions

We have demonstrated that amiodarone increased serum desmosterol level independently of gender, age, BMI, cardiac and other diseases, and lipid-lowering medication. Lipid analyses in patient samples taken before and after initiation of amiodarone showed a systematic increase of desmosterol upon drug administration, strongly arguing for a direct causal link between amiodarone and desmosterol accumulation. Mechanistically, we found that amiodarone resulted in desmosterol accumulation in cultured human cells and that the compound directly inhibited the DHCR24 enzyme activity in microsomal membranes.

Author Contribution

Piia Simonen: Conceptualization (lead); Investigation (lead); Project administration (lead); Software (lead); Writing-original draft (lead). **Shiqian Li:** Conceptualization (equal); Investigation (lead); Methodology (lead); Writing-review & editing (equal). **Ngee Kiat Chua:** Conceptualization (equal); Investigation (lead); Methodology (lead); Writing-review & editing (equal). **Anna-Maija Lampi:** Conceptualization (equal); Investigation (lead); Methodology (lead); Writing-review & editing (equal). **Vieno Piironen:** Conceptualization (equal); Investigation (lead); Resources (lead); Writing-review & editing (equal). **Jyri Lommi:** Conceptualization (equal); Investigation (lead); Project administration (equal); Resources (lead); Writing-review & editing (equal). **Juha Sinisalo:** Conceptualization (equal); Funding acquisition (lead); Investigation (lead); Resources (lead); Writing-review & editing (equal). **Andrew Brown:** Conceptualization (lead); Investigation (lead); Methodology (lead); Resources (lead); Writing-review & editing (lead). **Elina Ikonen:** Conceptualization (lead); Investigation (lead); Methodology (lead); Resources (lead); Writing-review & editing (lead). **Helena Gylling:** Conceptualization (lead); Investigation (equal); Methodology (lead); Project administration (equal); Validation (lead); Writing-review & editing (lead).

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Correspondence: Helena Gylling, MD, PhD, Biomedicum Helsinki 1 A4 23b, P.O. BOX 700, 00029 HUS, Helsinki, Finland. (e-mail: helena.gylling@hus.fi).■