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Regular Article

Rapid Stimulating Effect of the Antiarrhythmic Agent Amiodarone on Absorption of Organic Anion Compounds

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Summary: In a clinical setting, changes in pharmacokinetics due to drug-drug interactions can often directly affect the therapeutic safety and efficacy of drugs. Recently, interest has been shown in drug-drug interactions in the intestine. It is now recognized that changes in the functions of drug transporters substantially influence the absorption of administered drugs from the intestine. Amiodarone (AMD) is a potent drug used in the treatment of serious supraventricular and ventricular tachyarrhythmias. Despite its potent pharmacological effects, its wide clinical use is precluded by drug-drug interactions. In this study, we characterized the transporter function between AMD and various compounds in human intestinal model Caco-2 cells. AMD significantly and rapidly increased the uptake of [^{3}H]estrone-3-sulfate (E-3-S) for 5 min. The apical-to-basal transport of [^{3}H]E-3-S was significantly increased by AMD. The AMD-stimulated [^{3}H]E-3-S uptake was inhibited by organic anion transporting polypeptide (OATP) substrates. Caco-2 cells treated with AMD showed increased OATP2B1 expression on the cell surface. AMD also increased the absorption of sulfobromophthalein (BSP), which is a typical organic anion compound, and the expression level of Oatp2b1 at the membrane in *in vivo* experiments. The results indicate that AMD induces OATP2B1/Oatp2b1 expression at the membrane in the intestine and enhances absorption of organic anion compounds.

Keywords: amiodarone; drug-drug interaction; organic anion transport; absorption; OATP2B1

Introduction

In a clinical setting, changes in pharmacokinetics due to drug-drug and drug-food interactions can often directly affect the therapeutic safety and efficacy of drugs. In absorption, distribution, metabolism and excretion (ADME), a change in metabolic clearance of a drug, particularly *via* cytochrome P450 (CYP)-mediated metabolism, has long been considered as the cause of many clinically important drug interactions. However, since emerging evidence indicates an important role of drug transporters in modulating ADME, much interest has been shown in transporter-based drug interactions. In particular, it seems noteworthy that changes in the functions of drug transporters substantially influence the absorption of administered drugs from the intestine. Organic anion transporting polypeptide (OATP) 2B1 is a major uptake transporter in the intestine.[1)] OATP2B1 transports various clinically used therapeutic agents, such as rosuvastatin, fluvastatin, and mesalazine.[1,2)] Thus, it is important for prevention of adverse events to understand drug interaction mediated by OATP2B1 in the absorption process.[3,4)]

Amiodarone (AMD), a benzofuran derivative with class III antiarrhythmic activity that is effective in controlling intractable cardiac arrhythmias,[5,6)] is a potent drug used in the treatment of serious supraventricular and ventricular tachyarrhythmias. Clinical evidence suggests that AMD has a role in reducing the relative risk for arrhythmic or sudden death and overall mortality in survivors

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of myocardial infarction and in heart failure patients.[7] Thus, AMD is an interesting agent because it is widely used and co-administered with many kinds of drugs. However, its wide clinical use and long-lasting administration are precluded by extensive adverse effects such as interstitial pneumonia or lung fibrosis and drug-drug interactions mediated by CYP3A4, CYP2C8 and P-gp.[7,8] Moreover, it is known that long-term treatment with AMD up-regulates the expression of renal Mdr1 and multidrug resistance associated protein 2 (Mrp2).[9] However, no information on alteration of drug transporter expression is currently available for acute AMD treatment.

It is well known that acute drug interaction occurs by a direct inhibitory effect of the coadministered drug on transporters or CYP enzymes. However, there is no information on the acute stimulating effect of the coadministered drug on transporters or CYP enzymes. In this study, we determined a new type of drug interaction with a drug transporter. We found that acute AMD treatment results in induction of OATP2B1/Oatp2b1 expression at the membrane and that absorption of OATP2B1/Oatp2b1 substrates is significantly enhanced. These findings propound a new aspect of drug interaction study.

Materials and Methods

Chemicals: AMD was kindly supplied by Taisho Pharmaceutical (Tokyo, Japan). [^{3}H]Estrone-3-sulfate (E-3-S) (specific activity: 57.3 Ci/mmol), [^{3}H]taurocholic acid (specific activity: 5.0 Ci/mmol), [^{14}C]cholic acid (specific activity: 48.6 mCi/mmol), [^{3}H]*p*-aminohippuric acid (specific activity: 4.9 Ci/mmol), [^{3}H]-mannitol (specific activity: 17.0 Ci/mmol) and [^{125}I]thyroxin (T_4) (specific activity: 969 Ci/mmol) were purchased from Perkin Elmer (Boston, MA). [^{3}H]Estradiol-17β-gruclonide (specific activity: 41.8 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]Methotrexate (specific activity: 6.20 Ci/mmol) and [^{3}H]carnitine (specific activity: 81 Ci/mmol) were purchased from Amersham Biosciences (Buckinghamshire, UK). Sulfobromophthalein (BSP) was purchased from MP Biomedicals, Inc. (Santa Ana, CA). All other reagents were of the highest grade available and used without further purification. AMD was dissolved in methanol (1% w/v final concentration) due to its poor solubility in water.

Cell culture: Caco-2 cells obtained from American Type Culture Collection (Rockville, MD) were maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ). These stock cells were subcultivated before reaching confluence. The medium consisted of Dulbecco's Modified Eagle's Medium (Sigma, Tokyo, Japan) supplemented by 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), 100 IU/mL penicillin–100 µg/mL streptomycin (Sigma), 1% nonessential amino acid (Gibco, Grand Island, NY) and 2 mM $_L$-glutamine (Gibco). Monolayer cultures were grown in an atmosphere of 5% CO_2–95% air at 37°C. The cells were given fresh growth medium every 2 days. When the Caco-2 cells had reached confluence, they were harvested with 0.25 mM trypsin and 0.2% EDTA (0.5–1 min at 37°C), resuspended, and seeded into a new flask.

Animals: Male Wistar rats, aged 6 weeks, were obtained from Jla (Tokyo, Japan). The rats were housed for at least 1 week (until reaching 240–350 g in body weight). The housing conditions were the same as those described previously.[10] During the acclimatization, the rats were allowed free access to food (Rodent laboratory diet® EQ 5L37, PMI Nutrition International, LLC, St. Louis, MO) and water. Animals were fasted for 16 h before all experiments. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the *Guide for Care and Use of Laboratory Animals*.

Uptake study in Caco-2 cell monolayers: The accumulation of radiolabeled compounds and BSP was measured using monolayer cultures grown in 24-well plates (Corning Costar Corp., Cambridge, MA). Caco-2 cells were seeded (1×10^5 cells/well) and cultured for 4–6 days. The incubation medium used for the uptake study was HBSS-MES buffer (pH 5.0) (25 mM $_D$-glucose, 137 mM NaCl, 5.37 mM KCl, 0.3 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.8 mM $MgCl_2$ and 10 mM MES). In Na^+-free medium, NaCl was replaced with choline chloride. In Cl^--free medium, NaCl was replaced with sodium gluconate. In Ca^{2+}-free medium, $CaCl_2$ was replaced with $MgSO_4$. In K^+-free medium, KCl and KH_2PO_4 were replaced with NaCl and Na_2HPO_4. After removal of the growth medium, cells were preincubated at 37°C for 10 min with 0.5 mL HBSS-MES buffer (pH 5.0). After removal of the incubation medium, 0.5 mL of incubation medium containing radiolabeled compounds or BSP was added. The monolayers were incubated for the indicated time at 37°C. AMD was added at the time of preincubation (preload) or at the time of incubation (simultaneous load). Each cell monolayer was washed rapidly twice with ice-cold incubation medium at the end of the incubation period. To quantify the radioactivity of radiolabeled compounds, the cells were solubilized in 1% sodium dodecyl sulfate (SDS)/0.2 N NaOH. The remainder of the sample was mixed with 5 mL of a scintillation cocktail (Amersham International, Amersham, UK) to measure the radioactivity. To quantify the BSP concentration, the cells were solubilized in 1% SDS/0.2 N NaOH to measure the absorbance. All of the uptake values were corrected against the protein content.

Transcellular transport across Caco-2 cell monolayers: Caco-2 cells were seeded (2×10^5 cells/well) in a 12-well Transwell™ (Corning Costar Corp.) and grown for 18–21 days. The cells were given fresh growth medium every day. Before the experiment, the integrity of Caco-2 cell monolayers was checked by measuring the trans-epithelial electrical resistance (TEER) value with a Milicell ERS volt/ohmmeter from Millipore (Bedford, MA). Only monolayers showing TEER values between 300 and 400 Ω cm^2 were used. Transcellular transport of [^{3}H]E-3-S was measured using monolayer cultures grown in a 12-well Transwell™. The incubation medium used for the transcellular transport study was HBSS-MES (pH 5.0) buffer. After removal of the growth medium from both sides of the monolayers, the cells were preincubated at 37°C for 10 min with HBSS-MES (pH 5.0) (12-well; 1.5 mL of the basal side and 0.5 mL of the apical side). After removal of the incubation medium, incubation medium containing [^{3}H]E-3-S was added to the apical side or the basolateral side. The monolayers were incubated at 37°C for 30 min. For transport measurements, aliquots of incubation medium on the other side were taken at specified times and samples were collected for immediate analysis.

RT-PCR analysis: Caco-2 cells were seeded (4×10^5 cells/well) and cultured for 4–6 days in 6-well plates (Corning Costar Corp.). RT-PCR was performed as described previously.[11] Total RNA was prepared from Caco-2 cells using an ISOGEN (Nippon Gene, Tokyo, Japan) and an RNase-Free DNase Set (QIAGEN, Tokyo, Japan). Single-standard cDNA was made from 2 µg total RNA by reverse transcription (RT) using a ReverTraAce (Toyobo, Osaka, Japan). Gene-specific primers for OATP1A2, OATP1B1,