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MRP1 mutated in the L_0 region transports SN-38 but not leukotriene C_4 or estradiol-17 (β-D-glucuronate)

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Abstract

Multidrug resistance protein 1 (MRP1) is an ATP-binding cassette transporter that confers multidrug resistance on tumor cells. Much convincing evidence has accumulated that MRP1 transports most substances in a GSH-dependent manner. On the other hand, several reports have revealed that MRP1 can transport some substrates independently of GSH; however, the importance of GSH-independent transport activity is not well established and the mechanistic differences between GSH-dependent and -independent transport by MRP1 are unclear.

We previously demonstrated that the amino acids W_{261} and K_{267} in the L_0 region of MRP1 were important for leukotriene C_4 (LTC_4) transport activity of MRP1 and for GSH-dependent photolabeling of MRP1 with azidophenyl agosterol-A (azidoAG-A). In this paper, we further tested the effect of W222L, W223L and R230A mutations in MRP1, designated dmL_0MRP1, on MRP1 transport activity. SN-38 is an active metabolic form of CPT-11 that is one of the most promising anti-cancer drugs. Membrane vesicles prepared from cells expressing dmL_0MRP1 could transport SN-38, but not LTC_4 or estradiol-17 (β-D-glucuronate), and could not be photolabeled with azidoAG-A. These data suggested that SN-38 was transported by a different mechanism than that of GSH-dependent transport. Understanding the GSH-independent transport mechanism of MRP1, and identification of drugs that are transported by this mechanism, will be critical for combating MRP1-mediated drug resistance. We performed a pairwise comparison

Abbreviations: ABC transporter, ATP-binding cassette transporter; ACNU, 3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea; ADM, adriamycin; AG-A, agosterol A; azidoAG-A, [^{125}I] 11-azidophenyl agosterol A; AZT, azidothymidine; BSO, buthionine sulfoximine; BSP, sulfobromophthalein; C, COOH; CDDP, *cis*-platinum(II) diammine dichloride; d4T, 2′,3′-didehydro-3′-deoxythymidine; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DTT, dithiothreitol; $E_2$17β-G, estradiol-17 (β-D-glucuronate); γGCS, γ-glutamylcysteine synthetase; IAARh123, iodoaryl azidorhodamine 123; IAAQ, *N*-{4-[1-hydroxy-2-(dibutylamino) ethyl] quinolin-8-yl}-4-azidosalicylamide; IACI, *N*-(hydrocinchonidin-8′-yl)-4-azido-2-hydroxybenzamide; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; mAMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; MDR, multidrug resistance; MRP1, multidrug resistance protein 1; MV, membrane vesicles; N, NH_2; NBD, nucleotide-binding domain; NNAL-*o*-glucuronide, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol-*o*-glucuronide; P-gp, P-glycoprotein; PMEA, 9-(2-phosphonylmethoxyethyl) adenine; PMEG, 9-(2-phosphonylmethoxyethyl) guanine; 3TC, 2′,3′-dideoxy-3′-thiacytidine; TM, transmembrane segment; TMD, transmembrane domain; VCR, vincristine; VP-16, etoposide; Rh123, rhodamine 123

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of compounds that are transported by MRP1 in a GSH-dependent or -independent manner. These data indicated that it may be possible to predict compounds that are transported by MRP1 in a GSH-independent manner.

Keywords: MRP1; SN-38; GSH; L_0; ABC transporter; Drug resistance; LTC_4; $E_2$17β-G

1. Introduction

Multidrug resistance (MDR) is a major obstacle to successful cancer chemotherapy and is mediated by MDR transporters [1]. Human multidrug resistance protein 1 (MRP1) is frequently over-expressed in MDR cells that do not express the P-glycoprotein (P-gp) transporter [2]. MRP1 is a member of the family of ATP-binding cassette (ABC) transporters [3,4]. Over-expression of MRP1 in cultured cells resulted in a reduction of drug accumulation and an increase in the ATP-dependent efflux rate of drugs. As an organic anion transporter, MRP1 actively transports a wide variety of diverse anionic compounds [3,4]. Leukotriene C_4 (LTC_4) is an endogenous substrate of MRP1 with the highest known affinity for MRP1 (K_m about 100 nM) [5]. By using the in vitro inside-out membrane vesicle system, it was also found that physiological concentrations of GSH stimulated the ATP-dependent transport of certain drugs such as vincristine (VCR) [5–7], adriamycin (ADM) [7,8] and aflatoxin B_1 [9] as well as certain endogenous hydrophilic anionic conjugates such as 3-α-sulfatolithocholyl taurine, estrone-3-sulfate [10] and a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol-*o*-glucuronide (NNAL-*o*-glucuronide) [11]. GSH is supposed to be transported as conjugates or co-transported with endo- and xenotoxins by MRP1, however, since GSSG (K_m = 100 μM) is a preferential substrate of MRP1 rather than GSH. Under the oxidative stress condition, MRP1 pumps out cytoplasmic GSSG and may protect thiol group of other proteins from GSSG reactivity or affect to GSH metabolism [6,12].

Agosterol A (AG-A) is a novel inhibitor of MRP1 function and reverses MRP1-mediated MDR [13,14]. We synthesized a photoaffinity analog of AG-A, [^{125}I] azidoAG-A, and showed that photolabeling of MRP1 with [^{125}I] azidoAG-A was dependent on GSH. We and other group demonstrated that the L_0 region of MRP1 was important for the GSH-dependent photolabeling of MRP1 with azdioAG-A as well as for the LTC_4 transport activity of MRP1 [15–17].

Some evidence exists that MRP1 may be able to transport some molecules in a GSH-independent manner. Thus, MRP1 expressing cells were reported to be resistant to calcein, special toxic peptides 4A6 and SN-38 even in the presence of buthionine sulfoximine (BSO), a γ-glutamylcysteine synthetase (γGCS) inhibitor [18–20]. Furthermore, MRP1 was photolabeled even in the absence of GSH with the quinoline-based drugs, *N*-{4-[1-hydroxy-2-(dibutylamino) ethyl] quinolin-8-yl}-4-azidosalicylamide (IAAQ), *N*-(hydrocinchonidin-8′-yl)-4-azido-2-hydroxybenzamide (IACI) and iodoaryl azidorhodamine 123 (IAARh123) [21–23]. However, the importance and mechanism(s) of GSH-independent transport by MRP1 are still unclear.

SN-38 is a metabolized active molecule of CPT-11 which is now widely used as an effective drug for treatment of cancers. We could not detect any glucuronide conjugated SN-38 by HPLC analysis of either KB/MRP1 cells or of the culture media [20]. These data suggested that SN-38 could be transported without glucuronization. However, we could not rule out the possibility that SN-38 might be transported in the presence of another, as yet unidentified, molecule that might not require covalent binding of SN-38 for transport.

In this study, we find that resistance to SN-38 in MRP1-expressing cells cannot be effectively reversed with AG-A or BSO; these data suggest that SN-38 can be transported without GSH, and demonstrate the importance of GSH-independent transport by MRP1. Using a mutant MRP1, dmL_0MRP1, we show that GSH-independent transport of SN-38 is clearly distinct from GSH-dependent transport of other drugs by MRP1.

2. Materials and methods

2.1. Materials

[^{125}I] NaI (3.7 GBq/ml, [6,7-^{3}H (*N*)] estradiol-17β-glucuronide (1.11 TBq/mmol) and [14,15,19,20-^{3}H (*N*)]-LTC_4 (5.4 GBq/mmol) were purchased from Perkin-Elmer Life Sciences (Boston, MA). The synthesis and use of [^{125}I] 11-azidophenyl agosterol A ([^{125}I] azidoAG-A) were described in a previous paper [15]. Cellfectin, competent DH10Bac *E. coli* cells and Lipofectamine were purchased from Invitrogen Corp. (Carlsbad, CA). G418 was purchased from Nacalai Tesque Inc. (Kyoto, Japan). MRPm6, a monoclonal antibody against MRP1 and Tetra-His, anti-His monoclonal antibody were purchased from Progen Biotechnick (Heidelberg, Germany) and Qiagen GmbH (Hilden, Germany), respectively [24]. SN-38 and CPT-11 were kindly provided by Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan) and Yakult Pharmaceutical Ind. Co. Ltd. (Tokyo, Japan). Other drugs and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Cell culture, membrane vesicle preparation and cytotoxicity assay

KB-3-1 parentel cells and KB/MRP cells, that are MRP1 transfected KB-3-1 cells, were cultured in Minimal Essen-