Roles of Sildenafil in Enhancing Drug Sensitivity in Cancer

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Abstract

The phenomenon of multidrug resistance (MDR) has decreased the hope for successful cancer chemotherapy. The ATP-binding cassette (ABC) transporter superfamily is the largest transmembrane family. The overexpression of ABC transporters is a major determinant of MDR in cancer cells both in vitro and in vivo. Unfortunately, until recently, most of the strategies used to surmount ABC-transporter–mediated MDR have had limited success. An ideal modulator of MDR would be one that has a low liability to induce toxicity and alter the pharmacokinetic profile of antineoplastic drugs. Sildenafil, an inhibitor of cGMP-specific phosphodiesterase type 5, was found to significantly reverse ABC-transporter–mediated MDR. Our results indicate that sildenafil has differential inhibitory effects on ABC transporters: It significantly decreases the efflux activity of ABCB1 and ABCG2, but has no significant effects on ABCB1. Emerging evidence indicates that sildenafil and other phosphodiesterase type 5 inhibitors may enhance the sensitivity of certain types of cancer to standard chemotherapeutic drugs. Cancer Res; 71(11); 3735–8. © 2011 AACR.

Introduction

Multidrug resistance (MDR) in cancer cells is the primary cause of cancer chemotherapy failure. One of the major determinants of the MDR phenotype is the overexpression of ATP-binding cassette (ABC) transporters, which include ABCB1 (P-glycoprotein/MDR1), ABCCs [multidrug resistance–associated proteins (MRPs)], and ABCG2 (BCRP/MXR/ABCP) (1). Membrane proteins in this superfamily share the ability to actively transport a wide variety of substrates, ranging from ions, sugars, amino acids, vitamins, lipids, and drugs to larger molecules, across the cellular membrane, although each transporter has its own substrate specificity (1). When these transporters are overexpressed in cancer cells, they pump out or extrude structurally and mechanistically different chemotherapeutic drugs, thereby lowering intracellular drug concentration and leading to an attenuated chemotherapeutic effect (2). An increasing amount of evidence is showing that in addition to contributing to drug resistance, ABC transporters also play an important role in tumorigenesis (3). The expression of specific ABC transporters is associated with tumor-initiating cells or cancer stem cells in several types of cancer, and their clinical relevance is now being widely studied (3). Ideally, inhibition of these transporters would lead to resensitization of MDR cancer cells or cancer stem cells to chemotherapeutic drugs, and allow for a more efficacious treatment of cancer patients. For >30 years, investigators have been making a significant effort to develop specific inhibitors/modulators that can reverse MDR in cancer cells. Typically, these compounds are classified as first-, second-, or third-generation inhibitors/modulators. The first-generation inhibitors/modulators are drugs that are used clinically and have well-established pharmacologic profiles, such as verapamil (4) and cyclosporine A (5). However, both verapamil and cyclosporine A were shown to be ineffective in clinical trials as a result of serious adverse effects produced by the dose required to significantly reverse MDR (6, 7). Subsequently, second-generation inhibitors/modulators, derivatives of the first-generation inhibitors/modulators with more potent inhibitory activity (e.g., SDZ PSC833 and valspodar), were developed and tested (8). Unfortunately, data from clinical trials indicated that SDZ PSC833 inhibited the metabolism and elimination of certain clinically used anticancer drugs, thereby increasing their plasma levels and producing toxicity (9). Investigators then derived third-generation inhibitors/modulators from the second-generation lead compounds on the basis of their pharmacophores by chemical modification using structure-activity relationships. Subsequent studies indicated that these compounds, including LY335979 (zosuquidar; ref. 10), GF120918 (elacridar; ref. 11), and MS-209 (dofequidar; ref. 12), had a high affinity (nanomolar) for ABC transporters. Although they showed promising activity in preclinical studies and in early clinical trials as potent and nontoxic inhibitors/modulators, most of these compounds were found to lack significant efficacy in late-phase clinical trials (13). In light of the aforementioned results, it is clear that there is still a need to develop and test efficacious and nontoxic inhibitors/modulators.

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Sildenafil, an inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5), is clinically used to treat erectile dysfunction and pulmonary arterial hypertension (World Health Organization Class I). Sildenafil binds to PDE5 and prevents the biotransformation of second messenger 3',5'-cGMP to 5'-GMP, thus increasing intracellular cGMP levels. It has been reported that high cGMP levels are associated with increased blood flow as a result of vascular smooth muscle vasodilation (14). Previously, cyclic nucleotide second messengers such as cGMP and cAMP were shown to be substrates with low micromolar affinity for ABCC4/MRP4 and ABCC5/MRP5. Furthermore, sildenafil significantly inhibits the efflux activity of ABCC4 and ABCC5 (15, 16). Our recent studies showed that sildenafil also inhibits the activity of ABC transporters such as ABCB1 and ABCG2, and reverses MDR in cancer cells mediated by these transporters (ref. 17; Fig. 1).

**Key Findings**

Recently, we examined the in vitro effect of sildenafil on ABCB1-, ABCC1-, and ABCG2-mediated MDR in cancer cells.
Our data indicate that sildenafil has differential effects on these three transporters. Cytotoxicity assays showed that sildenafil significantly sensitized ABCB1-overexpressing cells to the ABCB1 substrates colchicine, vinblastine, and paclitaxel. In addition, sildenafil sensitized wild-type or mutant ABCG2-overexpressing cells to the ABCG2 substrates flavopiridol, mitoxantrone, and SN-38. However, sildenafil did not significantly sensitize ABCG2-overexpressing cells to its substrate, vincristine. In addition, sildenafil did not have any significant effect on the sensitivity of any of the parental cell lines to the aforementioned antineoplastic drugs. Consistent with the cytotoxicity data, the results of the drug accumulation study showed that sildenafil significantly enhanced the intracellular accumulation of paclitaxel in the ABCB1-overexpressing cells, as well as mitoxantrone and BODIPY-prazosin in either wild-type or mutant ABCG2-overexpressing cells. In addition, the results of membrane-vesicle transport experiments showed that sildenafil directly inhibited the ABCG2-mediated transport of E17βG and methotrexate. Sildenafil significantly stimulated ABCB1 and ABCG2 ATPase activity, whereas it inhibited photolabeling of ABCB1 and ABCG2 with [3H]IAAP. We also predicted the binding conformation of sildenafil within the large cavity of the transmembrane region of ABCB1 on the basis of the homology model (17).

We also examined the effect of another PDE5 inhibitor, vardenafil, a structural analogue of sildenafil, on ABC-transporter–mediated MDR in cancer cells. The results showed that vardenafil significantly sensitized ABCB1-overexpressing cells to the ABCB1 substrates vinblastine and paclitaxel, increased the intracellular accumulation of paclitaxel in the ABCB1-overexpressing cells, significantly stimulated the ATPase activity of ABCB1, and inhibited photolabeling of ABCB1 with IAAP. In contrast, vardenafil had no significant effect on any of the parental cells or on reversing ABCG2-mediated MDR (18).

Implications

Previous studies reported that increased PDE5 expression occurs in multiple human carcinomas, including urinary bladder cancers (19), metastatic breast cancers (20), and non–small cell lung cancers (21). These findings suggest that PDE5 may play a role in tumorigenesis. Therefore, inhibition of PDE5 activity may have antineoplastic effects. Several groups have evaluated the effect of sildenafil and other PDE5 inhibitors on cancer treatment. In one study (22), sildenafil and vardenafil suppressed tumor cell growth and induced caspase-dependent apoptosis of B-cell chronic lymphocytic leukemia cells in vitro. In a rat brain tumor model, the PDE5 inhibitors sildenafil and vardenafil increased the transport of doxorubicin across the blood–brain tumor barrier and enhanced the efficacy of chemotherapy (23). It was also shown that sildenafil reverses tumor-induced immunosuppression and enhances the anti-tumor response by reducing myeloid-derived suppressor cell function, leading to delay in tumor growth (24). Moreover, sildenafil was reported to enhance the sensitivity of breast cancer cells to doxorubicin without exacerbating its toxicity to either bone marrow cells or macrophages (25). Sildenafil also increased the chemotherapeutic efficacy of doxorubicin in prostate cancer in vivo and ameliorated cardiac dysfunction (26). Another PDE5 inhibitor, sulindac sulfide, selectively inhibits growth and induces apoptosis of human breast tumor cells by elevating cGMP and activating protein kinase G (PKG; ref. 27). On the basis of these studies and our data, it is reasonable to hypothesize that sildenafil may enhance the sensitivity of anticancer drugs and potentially improve the chemotherapeutic outcome in cancer patients due to its inhibitory effect on PDE5, ABCB1, and ABCG2. Future studies examining the combined use of sildenafil with anticancer drugs need to consider several issues. First, it is important to examine the expression levels of PDE5, ABCB1, and ABCG2 in cancer tissues. In addition to overexpression of ABC transporters, cancer cells feature other drug-resistance determinants, including changes in metabolizing and detoxifying systems, such as DNA repair and the cytochrome P450 oxidases, and drug-induced alterations in apoptosis (1). Hence, the expression levels of sildenafil target proteins such as PDE5, ABCB1, and ABCG2 would significantly determine the efficiency of sildenafil. Second, a determination of the concentrations that would be effective in vivo would definitely improve the outcome of the combined use of sildenafil with anticancer drugs. The maximum observed plasma concentration (C_{max}) of a single oral dose of 25 to 200 mg of sildenafil in healthy male subjects is 127 to 1,150 ng/mL (0.2–2 μmol/L; ref. 28), which is slightly lower than the concentration we observed for MDR reversal (17). Therefore, concentrations of sildenafil exceeding those required for PDE5 inhibition seem to be required to enhance the effects of chemotherapeutic drugs. Third, the pharmacokinetic profiles of sildenafil and anticancer drugs may affect each other, potentially resulting in an increased therapeutic response as well as adverse effects. This is possible because ABCB1 and ABCG2 are highly expressed in several normal tissues (1) where the concentration and distribution of sildenafil and anticancer drugs may be altered if the drugs are used in combination. Finally, sildenafil is primarily metabolized by the cytochrome P450 (CYP) isoenzyme CYP3A4 (29), and the substrates of CYP3A4 considerably overlap those of ABCB1 (29). Consequently, the metabolism and elimination of both sildenafil and anticancer drugs, some of which are substrates of both CYP3A4 and ABCB1 (30), may be affected when these drugs are used in combination.

Future Directions

Our studies show for the first time that 2 imidazotriazinone compounds, sildenafil and vardenafil (both of which are PDE5/PDE6 inhibitors), can reverse ABCB1- or ABCG2-mediated MDR in cancer cells by directly blocking their drug efflux function. In addition, our results suggest that imidazotriazine compounds may be a novel class of ABC transporters inhibitors. The effects of additional imidazotriazine compounds on ABC transporter function will be investigated in the future. Elucidating the structure-activity relationship involved in the inhibition of cGMP hydrodase and ABC transporters by sildenafil may allow for the synthesis of sildenafil analogues that are more efficacious for reversing MDR.
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