Minireview

Mechanisms and strategies to overcome multiple drug resistance in cancer

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Abstract One of the major problems in chemotherapy is multidrug resistance (MDR) against anticancer drugs. ATP-binding cassette (ABC) transporters are a family of proteins that mediate MDR via ATP-dependent drug efflux pumps. Many MDR inhibitors have been identified, but none of them have been proven clinically useful without side effects. Efforts continue to discover not toxic MDR inhibitors which lack pharmacokinetic interactions with anticancer drugs. Novel approaches have also been designed to inhibit or circumvent MDR. In this review, the structure and function of ABC transporters and development of MDR inhibitors are described briefly including various approaches to suppress MDR mechanisms.
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Keywords: Multidrug resistance; ATP-binding cassette transporters; Cancer chemotherapy; Multidrug resistance inhibitors

1. Introduction

The failure of the curative treatment of cancer patients often occurs as a result of intrinsic or acquired drug resistance of the tumor to chemotherapeutic agents. The resistance of tumors occurs not only to a single cytotoxic drug used, but also occurs as a cross-resistance to a whole range of drugs with different structures and cellular targets. This phenomenon is called multiple drug resistance (MDR). Once MDR appears, using high doses of drugs to overcome resistance is ineffective, toxic effects appear and resistance are further stimulated. Multidrug resistance (MDR) severely limits the effectiveness of chemotherapy in a variety of common malignancies and is responsible for the overall poor efficacy of cancer chemotherapy [1–5].

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The cytotoxic drugs that are most frequently associated with MDR are hydrophobic, amphipathic natural products, such as the taxanes (paclitaxel and docetaxel), vinca alkaloids (vinorelbine, vincristine, and vinblastine), anthracyclines (doxorubicin, daunorubicin, and epirubicin), epipodophyllotoxins (etoposide and teniposide), antimetabolites (methorexate, fluorouracil, cytosar, 5-azacytosine, 6-mercaptopurine, and gemcitabine) topotecan, dactinomycin, and mitomycin C [4,6–8].

Overexpression of ATP-binding cassette (ABC) transporters has been shown to be responsible for MDR [5]. Therefore elucidation of the structure and function for each ABC transporter is prerequisite for understanding how these transporters work and for reversing MDR. One strategy for reversal of MDR in cells expressing ABC transporters is combined use of anticancer drugs with chemosensitizers. Inhibitors of ABC transporters can be used to enhance oral bioavailability or the brain penetration of various drugs. Downregulation of MDR transporters and circumventing MDR mechanisms are other approaches to overcome MDR [3,5].

2. ATP-binding cassette (ABC) transporters

Identifying the mechanisms leading to intrinsic or acquired multidrug resistance (MDR) is important in developing more effective therapies [2]. The drug resistance in cancer cells often results from elevated expression of particular proteins, such as cell-membrane transporters, which can result in an increased efflux of the cytotoxic drugs from the cancer cells, thus lowering their intracellular concentrations [4,6,9]. The resistance mechanism is called typical or classical MDR when overexpression of the membrane efflux pumps is involved in MDR [5]. The classical MDR is due mostly to increased efflux pumps in the cell membrane of cells pumping anticancer drugs out of cells [1–3,5].

ATP-binding cassette (ABC) transporters are a family of transporter proteins that contribute to drug resistance via adenosinetriphosphate (ATP)-dependent drug efflux pumps [10]. Up to date, more than 100 ABC transporters from prokaryotes to humans and 48 human ABC genes have been identified that share sequence and structural homology [4,5,10,11]. Not more than 10 of the ATP transporters are reported to confer the drug-resistant phenotype [4,9]. The functions of 16 genes have been determined and 14 genes are related with several diseases present in humans [5,11,12]. Although the resistant proteins belong to the ABC superfamily, they are quite different with respect to gene locus, amino acid sequence, structure and substrate [5]. ABC proteins are present in all

known living species, with a relatively conserved structure which contains a combination of conserved ABC and trans-membrane domains (TMDs). In mammals, the functionally active ABC proteins consist of at least four of such domains, two TMDs and two ABCs. These domains may be present within one polypeptide chain (full transporters), or within two separate proteins (half-transporters). In this latter case, functional ABC transporters need the dimerization of specific half-transporters [13]. High sequence homology in the ATP-binding domains known as nucleotide-binding folds allows identification and classification of members of the ABC transporter family [10]. These transporters use the energy released from the hydrolysis of ATP to drive the transport of various molecules across the cell membrane [4,11]. They are involved in the transport of many substances, including the excretion of toxins from the liver, kidneys, and gastrointestinal tract. It has been increasingly recognized that transporter-mediated processes significantly modulate drug absorption, distribution, metabolism and excretion [13]. In addition to their physiologic expression in normal tissues, many are over-expressed, in human tumors [4]. There are seven subfamilies classified as ABC transporters (ABC-A through ABC-G) that are expressed in both normal and malignant cells [10]. A number of ABC transporters and the chemotherapy drugs to which they have been shown to confer resistance are listed in Table 1.

3. Structure and function of ABC transporters

The most typical efflux pump in the cell membrane is P-glycoprotein (P-gp) having the molecular weight of 170 kDa, due to the gene amplification of the normal human gene, ABCB1 (MDR1) gene [5,10]. P-gp belongs to the ATP-binding cassette (ABC) family of transporters. P-gp is responsible for transporting various xenobiotics (not limited to anticancer drugs) out of cells by using ATP. P-gp is glycosylated at the first extracellular loop and composed of 12 hydrophobic transmembrane domains (TMDs) and 2 nucleotide-binding domain (NBD). One NBD connects two TMDs with a hydrophilic NBD loop. TDMs form channels for substrate drugs, and efflux substrate drugs whereas NBDs are located in the interior of cytoplasm, and participate in ATP binding and hydrolysis [5,14]. Upon binding of ATP to the NBDs, P-gp undergoes conformational changes and the TMDs reorganize into three compact domains [5,15]. This reorganization opens the central pore and allows transport of hydrophobic drugs (transport substrates) directly from the lipid bilayer into the central pore of the transporter [5,16].

The expression of P-gp is usually highest in tumors that are derived from tissues that normally express P-gp, causing resistance to some cytotoxic agents before chemotherapy is initiated. In some tumors, the expression of P-gp may be low before chemotherapy, but is induced after chemotherapy, resulting in the development of MDR [4]. The failure of certain chemotherapeutic agents are believed to link to the rapid expression of P-gp [4,17]. For a long time, P-gp was believed to be the only protein capable of conferring MDR in mammalian tumor cells. However, several reports on human tumor cell lines displaying MDR in the absence of P-gp overexpression, together with studies that failed to detect P-gp in a variety of human tumors pointed to the existence of other MDR conferring proteins. In 1992, Susan Cole and Roger Deeley observed

amplification and increased expression of a novel gene, the MRP1 (ABCC1) (MDR related protein) gene in a non-P-gp MDR cell line, namely a small-cell lung carcinoma cell line [10,18]. Overexpression of MRPs, other ABC proteins, causing MDR in mammalian cells has been also observed in several other non-P-gp MDR cell lines. MRP1 has 3 membrane spanning domains, 2 nucleotide binding domains and extracellular N-terminal. Both the structure and drug resistance spectra of MRP1 and P-gp are similar except taxanes which are poor substrates for MRP1. The second member of the multidrug resistance protein (MRP) (ABCC) family is called the canalicular multi-organic anion transporter (CMOAT, MRP2) which is involved in bilirubin glucuronide transport and confers resistance to MRP1 substrates and cisplatin. MRP3 is expressed in liver and involved in the efflux of organic anions from the liver into the blood in case of biliary obstruction. MRP4 and MRP5 transport nucleosides and confer resistance to antiretroviral nucleoside analogs. MRP6 is a lipophilic anion pump with a wide spectrum of drug resistance. Among the members of MRP family, only MRP1 has been widely accepted to cause clinical drug resistance [10].

The latest ABC transporter involved in MDR was cloned by Ross and Doyle in 1998 from a mitoxantrone-resistant subline of the breast cancer cell line MCF-7/Adr/Vp [19]. It is part of ABCG subfamily. ABCG2 is the mitoxantrone resistance gene, breast cancer resistance protein (BCRP), or ABC transporter in placenta (ABC-P). BCRP is a half-transporter that probably acts as a homo- or heterodimer in transporting cytotoxic agents [10]. A 110 kDa protein originally named lung resistance-related protein/major vault protein (LRP/MVP) is not an ABC transporter, but a major vault protein which constitutes >70% of subcellular ribonucleoprotein particles called vaults. It is found in the cytoplasm and nuclear membrane, not on the cell membrane like P-gp and MRP and excludes drugs away from the nucleus into the cytoplasm. LRP is often associated with vesicles and lysosomes and function with drug sequestration into vesicles. After entering into vesicles, drugs are excluded from the cell by exocytosis [8]. LRP overexpression predicts a poor response to chemotherapy in acute myeloid leukaemia and ovarian carcinoma [10].

4. Development of MDR inhibitors

There are many studies to overcome MDR by inhibiting MDR transporters, to suppress or circumvent MDR mechanisms. The use of anticancer drugs that could escape from the ABC transporters might be a solution to avoid drug resistance. Anticancer drugs which are not the substrates of ABC transporters are alkylating drugs (cyclophosphamide), antimetabolites (5-fluorouracil), and the anthracycline modified drugs (annamycin and doxorubicin-peptide) [5].

Another method to overcome resistance to anticancer drugs is to administer compounds that would not be toxic themselves, but would inhibit ABC transporters [3,5]. The compounds that would reverse resistance against anticancer drugs are called MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers. They may modulate more than one transporter [3,5].

Clinical trials helped to unravel the problems associated with combination chemotherapy of anticancer drug(s) together with an MDR inhibitor. The first factor to be determined before

Table 1
Major ABC transporters associated with MDR, chemotherapy substrates and MDR inhibitors common other systematic substrates inhibitors

Common name	Other names	Systematic name	Substrates	Inhibitors
P-gp	MDR1	ABCB1	Adriamycin Actinomycin-D Bisantrene Daunorubicin Docetaxel Doxorubicin Etoposide Epirubicin Homoharringtonine Mitoxantrone Paclitaxel Teniposide Topotecan Vinblastine Vincristine Vinorelbine VP-16	Anthranilamide Cyclosporine D NSC-38721 (mitotane) Pipecolinate Quinoline OC-144-093 PSC-833 (valspodar) MS-209 LY-335979 (zosoquidar) XR-9576 (tariquidar) R-101933 (laniquidar) VX-710 (biricodar) GF-120918 (elacridar) ONT-093 Isothiocyanates Diallyl sulfide PK11195 Amooranin siRNA tRA 98006 Agosterol A Flavonoids
MRP1	–	ABCC1	Doxorubicin Daunorubicin Etoposide Epirubicin Methotrexate Paclitaxel Vincristine Vinorelbine	MS-209 XR-9576 (tariquidar) VX-710 (biricodar) Isothiocyanates tRA 98006 Agosterol A Rifampicin NSAIDs
MRP2	CMOAT	ABCC2	Cisplatin CPT-11 (irinotecan) Doxorubicin Etoposide Methotrexate Mitoxantrone Vincristine Vinblastine SN-38	XR-9576 (tariquidar) VX-710 (biricodar) Isothiocyanates tRA 98006
BCRP	MXR1, ABC-P	ABCG2	Bisantrene Camptothecin Daunorubicin Doxorubicin Epirubicin Flavopiridol Mitoxantrone SN-38 Topotecan CPT-11 (irinotecan)	GF-120918 (elacridar) tRA 98006 Flavonoids Phytoestrogens Imatinib mesylate Fumitremorgin C TAG- 139

Data from http://www.nutrigene.4t.com/humanabc.htm.
Liscovitch and Lavie [3]; Thomas and Coley [4]; Choi [5]; Ambudkar et al. [6], Stavrovskaya [8], Gottesman et al. [9]; Leonard et al. [10].

embarking a clinical trial is to identify the ABC transporter protein involved in drug resistance and to utilize an anticancer drug that would benefit from inhibition of that transporter protein. The anticancer drug(s) utilized should match the transporter protein being inhibited. The second factor is to monitor the plasma concentrations and in vivo effectiveness of the tested MDR inhibitor in order to verify that an effective inhibitory concentration was in fact achieved in vivo. The pharmacokinetic interaction between the anticancer drug(s) and the MDR inhibitor must be searched and avoided to prevent a reduction in anticancer drug dosage.

5. First-generation MDR modulators

Inhibiting P-gp and other ABC transporters has been extensively studied for more than two decades [3,4]. Many agents of diverse structure and function that modulate MDR have been identified, including calcium channel blockers (e.g., verapamil), calmodulin antagonists, steroidal agents, protein kinase C inhibitors, immunosuppressive drugs (e.g., cyclosporine A), antibiotics (e.g., erythromycin), antimalarials (e.g., quinine), psychotropic phenothiazines and indole alkaloids (e.g., fluphenazine and reserpine), steroid hormones and

anti-steroids (e.g., progesterone and tamoxifen), detergents (e.g., cremophorEL) and surfactants [4,20,21].

First-generation MDR drugs had other pharmacological activities and were not specifically developed for inhibiting MDR. Their affinity was low for ABC transporters and necessitated the use of high doses, resulting in unacceptable high toxicity which limited their application [4,7,20]. Clinical trials with first-generation MDR drugs failed for various reasons, often due to side effects [3,4,7,20,22]. Many of the first-generation chemosensitizers were themselves substrates for ABC transporters and competed with the cytotoxic drugs for efflux by the MDR pumps. Therefore, high serum concentrations of the chemosensitizers were needed to produce sufficient intracellular concentrations [6]. These limitations prompted the development of new chemosensitizers that are more potent, less toxic and selective for the P-gp and other ABC transporters [4,7].

6. Second-generation MDR modulators

Second-generation chemosensitizers were designed to reduce the side effects of the first generation drugs. Second-generation MDR modulators have a better pharmacologic profile than the first-generation compounds, still they retain some characteristics that limit their clinical usefulness. Co-administration of an MDR modulator usually elevate plasma concentrations of an anticancer drug by interfering its clearance or inhibiting its metabolism and excretion, thus leading to unacceptable toxicity that necessitates chemotherapy dose reductions in clinical trials down to pharmacologically ineffective levels [3,4]. The affinity of second-generation MDR drugs towards ABC transporters was too low to produce significant inhibition of MDR in vivo at tolerable doses [20].

Many of the anticancer drugs are substrates both for ABC transporter proteins and for the cytochrome P450 isoenzyme 3A4. Most of the second-generation MDR chemosensitizers are also substrates for cytochrome P450 3A4 and metabolized by this enzyme [4]. The competition between anticancer agents and MDR modulators for cytochrome P450 3A4 activity may result in unpredictable pharmacokinetic interactions. Co-administration of a MDR drug may significantly elevate plasma concentrations of an anticancer drug by interfering with its clearance (e.g., via biliary elimination) or metabolism (e.g., via the cytochrome P450 system). This would increase the concentration of an anticancer drug leading to unacceptable side effects, necessitating dose reductions down to pharmacologically ineffective levels [3]. However since the pharmacokinetic interactions between chemosensitizers and cytotoxic agents are unpredictable, reducing the dose of a cytotoxic agent may result in under- or over-dosing in patients [4,9,23]. The unpredictable effects of second-generation MDR modulators on cytochrome P450 3A4-mediated drug metabolism limits the use of these second-generation modulators in the treatment of multidrug resistance.

ABC transporters have well defined physiologic roles, often involving the elimination of xenobiotics, in regulating the permeability of the central nervous system (blood–brain barrier), the testes, and the placenta, thus preventing these systems from being exposed to cytotoxic agents circulating in the blood [4]. Most of the second-generation MDR chemosensitizers are substrates for ABC transporter family. Inhibition of these transporters could lessen the ability of normal cells and tissues to protect themselves from cytotoxic agents. Inhibition of non-target transporters may enhance adverse effects of anticancer drugs. Side effects due to modulation of MDR protein in normal tissues, especially blood–brain barrier should be monitored carefully to avoid neurological responses. Because of these problems, MDR inhibitors have not improved the therapeutic efficiency of anticancer drugs unless such agents lack significant pharmacokinetic interactions [3,24].

7. Third-generation MDR modulators

Third-generation molecules have been developed to overcome the limitations of the second generation MDR modulators [4,7]. They are not metabolized by cytochrome P450 3A4 and they do not alter the plasma pharmacokinetics of anticancer drugs. Third-generation agents specifically and potently inhibit P-gp and do not inhibit other ABC transporters [4]. None of the third-generation agents tested so far have caused clinically relevant alterations in the pharmacokinetics of the co-administered anticancer drugs. Because of their specificity for P-gp transporters and lack of interaction with cytochrome P450 3A4, third-generation P-gp inhibitors offer significant improvements in chemotherapy without a need for chemotherapy dose reductions [4].

Several such compounds are currently undergoing clinical trials in several cancer types [3]. A non-immunosuppressive cyclosporin D derivative (PSC-833; Valspodar; Novartis AG) was the first of these drugs to be studied [3]. Unfortunately, further research with PSC-833 revealed pharmacokinetic interactions with several anticancer drugs and inhibition of non-MDR-related transporters [3]. Due to these results, development of PSC-833 was discontinued [3].

Schering AG has developed a quinolone derivative MDR modulator (MS-209). It is used in combination with the anticancer drug (doxetaxel) in advanced solid (breast and lung cancer) tumors [3].

One of the most promising third-generation P-gp inhibitors is an anthranilamide derivative tariquidar (XR9576) which is developed by NCI/Xenova/QLT Company [3]. In phase I and II studies with paclitaxel and vinorelbine in ovarian cancer, tariquidar gave successful results and phase III trials have been initiated with tariquidar in patients with non-small cell lung cancer [3]. It binds specifically and non-competitively to the P-gp pump with a high affinity and potently inhibits the activity of the P-gp transporter [3,4,24,25]. Tariquidar inhibits the ATPase activity of P-gp [4,24]. Tariquidar is more potent and its inhibitory action on the P-gp transporter pump lasts longer in comparision to the effects of first- and second- generation P-gp modulators. In none of the clinical trials, tariquidar caused alterations in the pharmacokinetics of the coadministered cytotoxic agents such as paclitaxel, vinorelbine, or doxorubicin in patients with solid tumors [3,4]. This allows the use of standard doses of these chemotherapeutic agents without the need for dose reduction.

Vertex Pharmaceuticals Inc. developed a pipecolinate analog, VX-710 (biricodar, Incel) which is a high-affinity P-gp and MRP inhibitor. VX-710 has no pharmacokinetic interactions with doxorubicin and is undergoing trials in solid tumors [3].

Laniquidar (R101933; NCI/EORTC Inc.) and the substituted diarylimidazole ONT-093 (Ontogen Inc.) are among the third generation P-gp inhibitors. They have a high po-

tency and specificity for the P-gp transporter despite having diverse chemical structures and origins [4,24,26]. R101933 and ONT-093 were shown to inhibit P-gp pump with no effect on the pharmacokinetics of docetaxel and paclitaxel [3,4,24,26].

The cyclopropyldibenzosuberane modulator LY335979 developed by Eli Lilly Inc. was shown to competitively inhibit the binding of vinblastine to P-gp [3,4]. LY335979 showed no significant pharmacokinetic interactions with doxorubicin, etoposide, daunorubicin, vincristine, or paclitaxel in both solid and hematologic malignancies [4,27,28].

Mitotane is not a typically third-generation drug and does not inhibit P-gp. It was utilized for treatment of adrenocortical carcinoma in combination with anticancer drugs [3]. GlaxoSmithKline developed GF-120918 (elacridar) which inhibits P-gp and BCRP and shows no pharmacokinetic interactions with doxorubicin. Annamycin (Antigenics Inc.) is not a P-gp inhibitor, but an anthracycline that is not transported by P-gp [3].

Clinical trials with these new third-generation agents are ongoing with the aim for a longer survival in cancer patients. This effort continues, but none of them has found a general clinical use so far.

8. Novel approaches to struggle with MDR mechanisms

The difficulties encountered with MDR inhibitors have led several alternative approaches to MDR therapy. These approaches can be divided in two groups. One group of studies consists of trials designed to inhibit MDR mechanisms in novel ways and the other group focuses on trials to circumvent MDR mechanisms [3].

There are several approaches to inhibit mechanisms involved in regulation of MDR transporters. MDR protein gene expression in tumor cells is induced upon treatment with cytotoxic drugs, whereas this gene expression is inhibited by several pharmacological inhibitors that affect the signaling pathways. It was demonstrated that taxol stimulated MDR1 and cytochrome p450 3A4 (*CYP3A4*) gene expression via its direct interaction with and activation of the nuclear steroid and xenobiotic receptor (SXR) which led to increased drug resistance and faster drug clearance [3]. Hence, antagonists of the nuclear steroid and xenobiotic receptor may be utilized in conjunction with anticancer drugs to cope with the induction of MDR1 and CYP3A4 [3].

Recent advances in antisense oligonucleotide technologies suggest an alternative and more specific way to cope with MDR than the use conventional MDR inhibitors [29]. Downregulation of ABC transporter proteins and enzymes involved in cancer cell resistance using antisense oligonucleotides may provide an efficient approach to overcome MDR.

Recent studies clarified the role of ceramide as a second messenger in cellular apoptotic signaling events [30]. A decrease in ceramide production increases cellular resistance to apoptosis. It was demonstrated that glucosylceramide (GC), a simple glycosylated form of ceramide which results from elevated GlcCer synthase activity accumulates in multidrug resistant cancer cells and tumors derived from patients who are less responsive to chemotherapy [31–33]. Overexpression of recombinant GlcCer synthase (GCS) confers resistance to adriamycin and to ceramide in GlcCer synthase-transfected human breast cancer

cells, suggesting that drug resistance is related to stimulation of glucosylation of ceramide and the resultant inhibition of drug-induced apoptotic signaling [30,34]. Blocking the glycosylation of ceramide has been shown to increase cancer cell sensitivity to cytotoxics [35–37]. Drug combinations that enhance ceramide generation and limit glycosylation have been shown to enhance effectiveness of chemotherapy by inducing apoptosis in cancer cell models [36,37]. The role of GlcCer synthase in drug resistance was demonstrated directly by antisense suppression of GlcCer synthase expression in MDR cells [30]. Downregulation of ceramide glycosylation using GCS antisense in adriamycin-resistant breast cancer cells restored cell sensitivity to adriamycin [30]. In another study, a novel amino-ceramide analog was shown to inhibit GlcCer synthase and thereby elevate ceramide production in MDR cells, enhancing drug-induced apoptosis [3]. These findings assign biological significance to ceramide metabolism and provides a promising approach to struggle with drug resistance. These results indicate that GlcCer synthase contributes to drug resistance in MDR cells by attenuating drug-induced formation of apoptotic ceramide and suggest that GlcCer synthase may represent a novel drug target in cancer MDR [38].

Living cells needs MDR mechanisms for their normal physiology. Therefore, researchers prefer to circumvent rather than directly inhibit MDR mechanisms [3]. Developing anticancer drugs that are poor substrates for ABC transporters might be a good strategy in cancer therapy. Another approach is to prevent formation of new blood vessels which is called angiogenesis. For a long time, it is believed that tumors induce angiogenesis to provide an adequate blood supply for oxygen and nutrients. New vessel formation are inhibited using anti-angiogenic factors. However, most of the anti-angiogenic factors have other effects on the cells which limit their treatment.

A major dose-limiting toxicity factor for anticancer drugs is to avoid complete eradication of bone marrow stem cells. Gottesman et al. produced multidrug resistant bone marrow cells by transfecting them with vectors carrying the MDR1 cDNA. This procedure allowed them to apply a chemotherapeutic regimen at otherwise unacceptable doses, and thus overcoming MDR [3,9].

Recent studies suggest that a mutant ABCG2 protein is an ideal candidate for human stem cell protection and for use as a selectable marker in gene therapy [13]. The cDNA encoding this protein is relatively small (about 2 kb) and the active dimer is spontaneously formed in the overexpressing cells. Since the R482G variant of ABCG2 has different substrate specificity than the wild-type protein, this mutant has a special advantage in gene therapy applications [13].

A novel procedure for circumventing MDR mechanisms, involves the use of an apoptosis inducing monoclonal antibody directed against the CD20 receptor. Induction of apoptosis in drug sensitive cells improves the efficacy of chemotherapy [39].

The exact mechanism of MRP1 involved multidrug resistance has not been clarified yet, though glutathione (GSH) is likely to have a role for the resistance to occur. *N*-acetylcysteine (NAC) is a pro-glutathione drug. DL-Buthionine (*S,R*)-sulfoximine (BSO) is an inhibitor of GSH synthesis. Recently, we investigated the effect of NAC and BSO on MRP1-mediated vincristine and doxorubicine resistance in human embryonic kidney (HEK293) and its MRP1 transfected 293MRP cells [1,2]. Human embryonic kidney (HEK293) cells were transfec-

ted with a plasmid encoding whole MRP1 gene. Both cells were incubated with vincristine and doxorubicine in the presence or absence of NAC and/or BSO. *N*-acetylcysteine increased the resistance of both cells against vincristine and doxorubicine. In contrast, BSO decreased NAC-enhanced MRP1-mediated resistance, indicating that induction of MRP1-mediated resistance depends on GSH. Our results indicate that NAC and BSO have opposite effects in MRP1 mediated vincristine and doxorubicine resistance and BSO seems a promising chemotherapy improving agent in MRP1 over-expressing tumor cells.

A number of drugs used in cancer chemotherapy induce oxidative stress by generation of reactive oxygen species (ROS). Recent studies suggest that ROS formation caused by these drugs might be an alternative mechanism for their cytotoxic effect via inducing apoptosis. Since ROS are proposed to play a role in drug-induced apoptosis, one might suspect that antioxidants would inhibit the ability of chemotherapeutic drugs to induce ROS generation and antioxidant supplementation should be avoided in cancer patients in order not to prevent chemotherapy induced apoptosis. Chemopreventive approaches utilizing non-toxic agents aimed at both minimizing ROS formation and inducing apoptosis in tumor cells seem attractive.

In a recent study it was demonstrated that novel catechin derivatives obtained from grape procyanidins scavenge free radicals, and reduce cell viability in A375 and M21 melanoma cells [40]. In particular, 4b-(*S*-cysteinyl) epicatechin 3-*O*-gallate has a free radical scavenging capacity and causes a significant S-phase cell-cycle arrest in both cell lines at doses higher than 100 μM. The gallate derivative also induces apoptosis in melanoma cells triggering nuclear condensation and fragmentation, which is confirmed by DNA laddering. In contrast, it does not induce apoptosis in keratinocytes (HaCaT) [40].

An impressive body of information exists on the antitumor action of plant flavonoids [41]. In vitro work has concentrated on the direct and indirect actions of flavonoids on tumor cells, and has found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and tumor invasive behavior. Furthermore, some studies have reported the impairment of in vivo angiogenesis by dietary flavonoids. Experimental animal studies indicate that certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the B ring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence their activities, especially the inhibition of protein kinase activity and antiproliferation [41]. In our recent study, the flavonoid, Quercetin (3,3′,4′,5,7-pentahydroxyflavone) did not inhibit ROS generation, and enhanced cytotoxicity of Topotecan in two human breast cancer cell lines, MCF-7 and MDA-MB-231 [42,43]. The different mechanisms underlying the potential anticancer action of plant flavonoids await further elucidation. Further in vivo studies of these bioactive constituents is necessary in order to develop flavonoid-based anticancer strategies. These studies demonstrate that antioxidants may have diverse effects in the cytotoxicity of chemotherapeutic drugs depending on their other pharmacological properties which may predominate their antioxidant effects.

9. Conclusion

In the last two decades, search for effective and clinically applicable MDR therapies took place to clarify the mechanisms underlying MDR and to develop agents either to inhibit or circumvent MDR mechanisms. Novel approaches are being devised to overcome MDR mechanisms. In spite of advances in cancer chemotherapy and developing a few good candidates to modulate MDR, we are still too far to conclude that these agents could be applied clinically. We may expect that advances in cancer chemotherapy will continue and knowledge to achieve effective chemotherapy will increase exponentially in the not too distant future.

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