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Flavonoids: An Emerging Lead in the P-glycoprotein Inhibition

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Abstract

Multidrug resistance is a major obstacle in cancer chemotherapy. Cancer cells efflux chemotherapeutic drug out of cell by means of transporter and reduce the active concentration of it inside cell. Such transporters are member of the ATP binding cassettes (ABC) protein. It includes P-gp, multiple resistant protein (MRP), and breast cancer resistant protein (BCRP). These proteins are widely distributed in the human cells such as kidney, lung, endothelial cells of blood brain barrier etc. However, there are number of drugs developed for it, but most of them are getting transported by it. So, still there is necessity of a good modulator, which could effectively combat the transport of chemotherapeutic agents. Natural products origin modulators were found to be effective against transporter such as flavonoids, which belongs to third generation modulators. They have advantage over synthetic inhibitor in the sense that they have simple structure and abundant in nature. This review focuses on the P-gp structure its architecture, efflux mechanism, herbal inhibitors and their mechanism of action.

Key words : Human P-gp, Anticancer, Flavonoids, NBD

1. Introduction

Multi drug resistance (MDR) is a major obstacle in cancer chemotherapy. MDR is defined as a resistance to a multiple unrelated drug when exposed to a single drug. MDR is mainly related to the over expression of the ATP-binding cassette (ABC) transporters. These proteins actively extrude a wide variety of structurally different substrates out of the tumor cells, thereby decreasing their effective intracellular concentrations. ABC transporter superfamily consist of 49 members in human and have been identified and classified into seven subfamilies (coded by the letters A to G) based on their phylogenetic similarity[1]. Among them, three proteins from the B, C, and G subfamilies have been primarily associated with the MDR phenomenon: P-glycoprotein, P-gp (ABCB1); the multidrug resistance-associated protein, MRP1 (ABCC1); and the breast cancer resistance protein, BCRP (ABCG2)[2,3]. These transporters can simultaneously be overexpressed in tumor cells, thus outlining MDR as a multi-factorial problem for the treatment of cancer. P-gp is most intensively studied among them. It is also known that the P-gp is distributed all over the human body such as kidney, lung, liver, blood brain barrier etc. Many compounds have been reported as a substrate and inhibitors of the P-gp which includes anticancer compounds from the natural origin such as taxol, vinca alkaloids and epipodophylotoxins. MRP is the second most studied ABC protein after P-gp and it is discovered in small cell lung cancer[4]. P-gp and MRP both shows resistance to a similar but not identical spectrum of cytotoxic drugs[5,6]. BCRP was first identified in the MCF-7/AdrVp cell line that does not express P-gp and MRP1[7-9]. The most surprising features of these MDR proteins are diversity of the recognized substrate which belongs to different chemical classes with no structural similarity[9].

2. P-gp Structure

In order to understand the molecular mechanism of P-gp mediated drug transport and in particular the determinant of substrate specificity, X-ray crystal structure of P-gp is necessary. Recently, three mouse P-gp structures were reported, which contain apo-protein and two struc-

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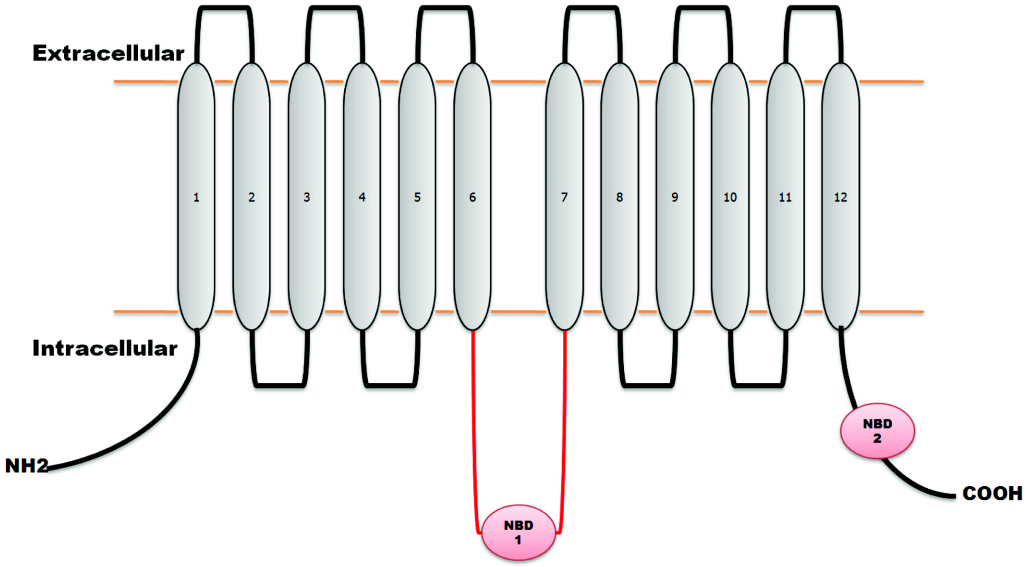


Fig. 1. General Architecture of P-gp.

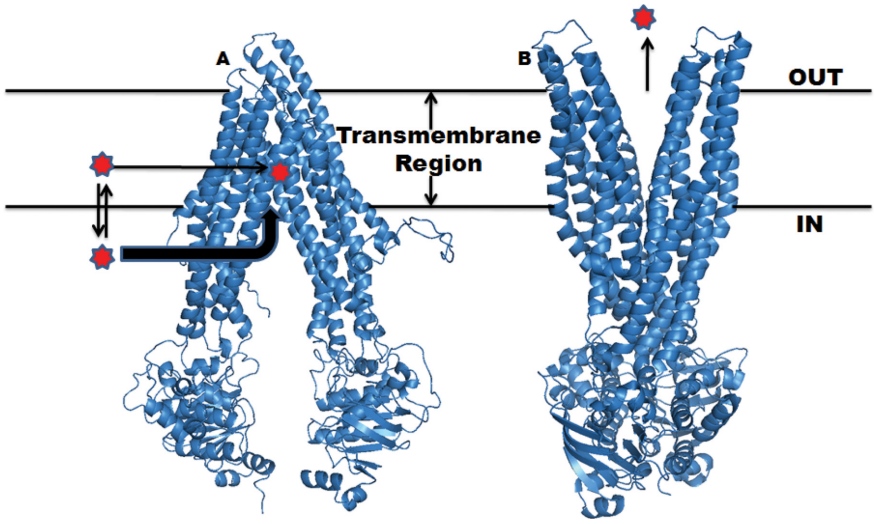


Fig. 2. Homology model of human P-gp in inward and outward facing conformation to show the mechanism of drug efflux.

tures complexed with the cyclic peptide inhibitors[10]. However, previously high resolution bacterial ABC transporter protein was reported, which is in outward conformation[11]. General architecture of P-gp is presented in Fig. 1. Human P-gp is a membrane protein consists of 1280 amino acids. This structure divided into four domains such as two hydrophobic transmembrane domain and two hydrophilic nucleotide binding domains (NBD). Half hydrophobic transmembrane domain and one NBD make half transporter, and it is believed that the half transporter is similar to other half, which suggests pseudo symmetry to protein structure. Each half transporter consists of 610 amino acids and linked by a linker of 60 amino acids. P-gp consists of 12 transmembrane helices, 6 in each half transporter. However, this linker region is highly charged and phos-

phorylated at several sites by protein kinase C (PKC) *in vivo* and *in vitro*[11]. Phosphorylation of this linker is not require for the active transport[12,13], but modulates ability of P-gp to regulate heterologous ion channels[14]. Previously reported two structures are in inward and outward facing conformations. It gives idea that during transport cycle the P-gp conformation changes from inward-outward or outward-inward (Fig. 2) to translocate substrate against concentration gradients with the help of ATP.

3. Drugs Substrates/Inhibitors in Cancer Cells

Since the discovery of the first P-gp inhibitor, verapamil[15], a lot of studies have been performed to understand the protein efflux function and to create specific and effective MDR inhibitors, so called MDR modulators. All the known MDR modulators are classified into three generations. To the first generation belong compounds already used clinically for other diseases (like verapamil, cyclosporin A, and quinidine). They showed high toxicity when applied in doses required for MDR reversal. It is known that these compounds are not effective at lower concentrations and to achieve therapeutic effect higher dose is necessary and hence high dose related high toxicity was observed. The intensive search for more specific and less toxic compounds led to the development of second generations of MDR inhibitors. Nowadays, the third generations of MDR modulators are in the focus of interest[16]. They represent novel molecules composed of structural features preselected on structure-activity relationships and then submitted to pharmacological screening[17]. In contrast to the second-generation MDR modulators, these inhibitors are not cytochrome P450 3A4 substrates, and do not influence significantly the pharmacokinetic profile of co-administered drugs[16,18]. Prominent members among the third-generation MDR modulators are elacridar (GF120918) and tariquidar (XR9576), both containing a dimethoxytetrahydroisoquinoline-ethyl-phenylamine partial structure. Tariquidar belongs to a series of compounds, called XR compounds, which have been developed by Xenova Group Ltd.[19]. A number of new tariquidar analogs have been synthesized and pharmacologically tested[20-22], thus supplying good data for a profound structure-activity investigation of this promising class of MDR modulators. However, number of natural origin and structurally unrelated compounds are the substrates of P-gp, which are extruded by the P-gp. Natural origin compounds such as vinca alkaloids, topotecan, epipodophylotoxins, taxanes are the substrates of P-gp.

4. Mechanism of Efflux

P-gp mediated efflux is ATP dependent which is against concentration gradient. The first step in drug efflux is most likely drug recognition by P-gp followed by ATP-binding and hydrolysis. Most of the drug binding sites are located into the residues from TM6, TM12, TM1, TM4, TM10, and TM11. There is the formation of a hydrophobic binding pocket which plays a role in determining the suitable substrate/drug size for P-gp and therefore substrate specificity[23]. The energy released in this process is utilized by transporter to efflux substrate outside the cell membrane through central pore. In contrast, the previous report[24] suggested that two ATP molecules are hydrolyzed for the transport of every substrate molecule; one molecule to extrude substrate and the other in causing conformational changes to reset the pump for the next catalytic cycle. After hydrolysis the release of ADP molecules from the nucleotide binding site ends the first catalytic cycle followed by a conformational change that reduces affinity for both substrate and nucleotide. Furthermore, the second catalytic cycle starts by hydrolysis of another molecule of ATP and released energy is utilized to reorient the protein to its native conformation. Subsequent release of ADP completes another catalytic cycle, bringing P-gp molecule back to the original state, where it again binds to both substrate and nucleotide to initiate the next cycle[25].

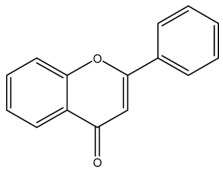
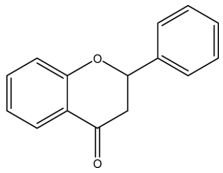
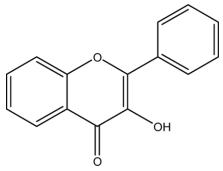
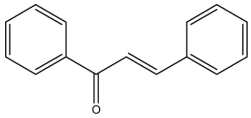
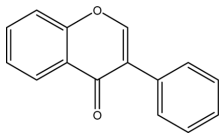
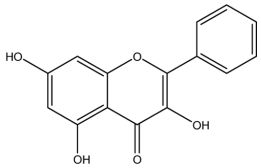
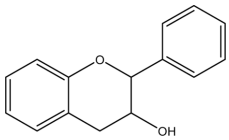
The mechanism of xenobiotic extrusion by P-gp has been described by various ways; however, the exact site of substrate interaction with the protein is not well resolved[26]. Because, it was reported that the P-gp contain different binding sites in an one translocation pore. And different drugs interact at different region of P-gp. The most popular models include pore model, flippase model and hydrophobic vacuum cleaner model[27]. However, hydrophobic vacuum cleaner model is widely accepted and gain popularity, which works by recognizing its substrate from the membrane leaflet and transport it through protein channel. It is so called

hydrophobic vacuum cleaner because most of the substrates of P-gp are hydrophobic with the multiple planar ring system and positively charged at normal physiological pH. Change in electric potential and pH across membrane may also contribute extrusion process.

5. Phytochemical Flavonoids as P-gp Modulators

Flavonoids are the constituents abundant in plants, vegetables, fruits and acts as P-gp modulators. Several beneficial properties have been attributed to flavonoids including antioxidant, antiinflammatory, and anticarcinogenic effects. Flavonoids consist of different skeleton and are reported in Table 1. Flavonoids possess a chromane ring skeleton with an additional aromatic ring attached at position 2, 3 or 4[28]. Based on the different substitution and the oxidation status of ring C, flavonoids can be classified into several subclasses including flavones, flavonols, flavonones, flavanols, isoflavones

Table 1. Flavonoids class, representative structures and their examples

Class	Structure	Examples
Flavones		Baicalein, luteolin, flavone, Apigenin, diosmetin, nobiletin, techtochrysin, diosmin, tangeretin, chrysin
Flavonones		Naringenin, naringin, hesperitin, eriodictoyl, hesperidin, pinocembrin, likvirtin
Flavonols		Isoquercetrin, kaemferol, morin, rutin, myricetin, quercetin, quercetrin, myricitrin, spiraeocide, galangin, robinin, kaempferide, fisetin, rhamnetin
Chalcones		Phloretin
Isoflavones		Genistein, daidzin
Flavonolols		Pinocembrin, silibinin, silymarin, taxifolin,
Flavan-3-ols		Acacetin, catechin, epi-catechin, epigallocatechin

and chalcones. Recently various flavonoids have been reported as P-gp transport inhibitors affecting the bioavailability and uptake of anticancer drugs. In general, P-gp can be inhibited by more than one mechanism. There could be blockade of drug binding site either competitively, non-competitive or allosterically[27]; interference with the ATP hydrolysis process[29,30]; alteration in integrity of cell membrane lipids[31] or decrease in P-gp expression[32]. Drugs such as cyclosporine-A inhibit transport function by interfering with both substrate recognition and ATP hydrolysis. Compounds inhibiting ATP hydrolysis could serve as better inhibitors, since they are unlikely to be transported by P-gp, and these kinds of agents require low dose which is achievable at target site. It has been shown that the flavonoids genistein, epicatechin gallate, catechin gallate, epigallocatechin gallate and silymarin can inhibit the labeling of P-gp with its photoactive substrates indicating that they can directly bind to substrate region. In addition, flavonoids have also been shown to directly interact with the purified recombinant C-terminal nucleotide-binding domain from mouse P-gp (NBD2), and this binding domain may overlap with the ATP binding site and vicinal steroid binding site. It is also known that increase in hydrophobicity of flavonoids shift flavonoids binding in an steroid binding site.

The SAR for flavonoid-P-gp interaction has been extensively studied by evaluating the binding affinity of different flavonoids with mouse NBD2 and reviewed by Boumendjel et al.[33]. Although most of the compounds inhibit P-gp function by blocking drug binding sites, presence of multiple binding sites complicate understanding as well as hinder developing a true, conclusive SAR for substrates or inhibitors.

6. Conclusion

P-gp has been found to be responsible for the MDR in humans. Number of modulators have been reported but later on found to be transported by P-gp itself. Flavonoids are the natural origin compounds and are highly effective to combat the transport of substrates, because of its site of action are different than the substrates. Flavonoids were found to be a promising leads to treat the cancer. They could bind to the NBD and make hindrance to ATP binding and hydrolysis, which power the transport of substrate out of cells.

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