Unveiling the Role of Multidrug Resistance Proteins in Hypertension

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Hypertension is a chronic condition associated with an increased risk of mortality and morbidity. It is estimated to cause 4.5% of current global disease burden, and its prevalence is similar in both developing and developed countries.1 According to a recent review, ≈54% of stroke, 47% of ischemic heart disease, 75% of hypertensive disease, and 25% of other cardiovascular disease worldwide can be attributable to high blood pressure.2

Altered levels of angiotensin and aldosterone are common findings in hypertension. Aldosterone has been shown to play an important role in the pathophysiology of numerous cardiovascular disorders, including heart failure and hypertension,3 and the renin-angiotensin-aldosterone system is currently an important target for 5 antihypertensive drug classes: β-blockers, renin inhibitors, angiotensin-conversion enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists.4

In the last few years, increasing evidence has pointed to an involvement of multidrug resistance (MDR)–related proteins with hypertension. Both ABCB1 (P-glycoprotein) and ABCC1 (MDR–associated protein 1), the 2 main proteins first described in multidrug-resistant tumors, are known to physiologically transport several endobiotics, including hormones.5,6 Moreover, ABCB1 and ABCG2 (the third protein related to MDR), seem to also be related to the secretion of several drugs to urine, including some antihypertensives.7,8

This review summarizes the recent findings regarding the relationship between MDR-related proteins and hypertension.

ABC Superfamily and MDR in Cancer

MDR is still the main cause of failure in cancer chemotherapy. Although it is a multifactorial phenomenon, virtually all MDR cells present an ATP-dependent reduction in intracellular drug accumulation because of the overexpression of ≥1 of 3 proteins belonging to the ABC-binding cassette (ABC) family of transporters.9,10

The ABC superfamily is one of the largest protein families, and its members have been found in virtually all organisms examined up to now. It is consisted of 7 subfamilies, ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG, in which each protein receives a number, eg, ABCA1, ABCC7, or ABCG2. The majority of the ABC proteins are membrane transporters that actively translocate a wide range of substrates to various cellular compartments using ATP hydrolysis, which may explain the many functions fulfilled by these proteins in different organisms.

The first ABC protein related to MDR was initially named P-glycoprotein and subsequently MDR1, with P being a reference for an apparent altered membrane permeability conferred by its overexpression.11 This protein is now called ABCB1, meaning that it is the first member of the B subgroup of the ABC superfamily. The second MDR-related protein was discovered 16 years later and was called MDR-associated protein,12 or MDR-associated protein 1, and is now renamed ABCC1. The third important MDR-related protein, now called ABCG2, was discovered almost simultaneously by 3 distinct groups, receiving the names breast cancer resistance protein, mitoxantrone resistance protein, and placenta ABC protein.13–15

The ABCB1, ABCC1, and ABCG2 proteins mediate the unidirectional flux of substances used in cancer chemotherapy from the cytoplasm out of the tumor cells (Figure), conferring resistance to a broad range of drugs.9–16 This has been the basis for the study of these proteins in cancer.

Physiological Roles of MDR-Related Proteins

Although the roles of ABCB1, ABCC1, and ABCG2 in MDR tumors have been extensively studied, their function and regulation in normal tissues are so far unclear. It is now commonly accepted that the mainly studied protein, ABCB1, is related to the transport of hormones and other endogenous molecules, eg, aldosterone and glucocorticoids,5,16 as well as to the protection against xenobiotics and endobiotics.9,10,17–20

This protein was detected in several normal tissues, eg, kidney, intestine, adrenal, liver, blood-brain barrier, and cells of the immune system.21,22

The presence of this protein in the apical membranes of renal tubular cells and intestinal absorptive cells interferes with drug availability, metabolism, and toxicity because it reduces intestinal absorption and increases renal secretion of several drugs and metabolites, reducing the bioavailability of a wide range of drugs that are substrates for this membrane...
shown that abcb1a caused by abnormalities in the epithelial lining of the gut.27 and signs of severe chronic inflammation of the gut, probably attributed to this protein seem to be related to inflammation and oxidative stress via the secretion of leukotriene C4 and oxidized glutathione. Other important molecules that have been shown to be exported by ABCC1 are the prostaglandins A2 and J2, substances also derived from the arachidonic acid degradation product of activation of the 5-lipoxygenase system for which the function is related to increased cellular permeability and oxidative stress, vascular smooth muscle cell migration, and arterial tone.35 In fact, the main physiological functions attributed to this protein seem to be related to inflammation and oxidative stress via the secretion of leukotriene C4 or the transport of both reduced glutathione and oxidized glutathione.

The second protein related to MDR, ABCC1, is expressed in the apical membrane of several organs, including the kidney and brain. It is highly expressed in the gastrointestinal tract and liver.40–41 (Figure). Like ABCB1, ABCG2 does not require glutathione for the transport activity,42 and there is considerable overlap in anticancer drug substrate specificity between these 2 proteins.43 However, ABCG2 is able to transport phase 2 metabolites, eg, drugs conjugated to sulfate or glutathione.44–47

ABCG2-deficient mice showed lethal phototoxicity when fed with an alfalfa-containing diet because of pheophorbide-A retention, suggesting that this protein is involved in porphyrin excretion.48 In addition, it has been reported that ABCG2 transports androgens and phytoestrogens49–51 and that a sex-dependent expression of ABCG2 in the liver may be a cause of sex-specific variability in the pharmacokinetics of substrates for this protein in both mice and humans.52 In addition, it has been shown that expression of ABCG2 may

transporter19,20 (Figure). Its expression in the blood-organ barriers, eg, the blood-brain barrier, hampers the entry of certain molecules into the central nervous system and placent-
a.23 ABCB1 is important as well for the adequate functioning of the immune system because it promotes the extrusion of cytokines. It is also involved in T-cell migration and in the development of γδ-T cells.16,24–26

Altered gastrointestinal and renal phenotypes were reported in abcb1-deficient mice. Rodents have 2 genes encoding for the efflux pumps, abcb1a and abcb1b. It has been shown that abcb1a−/− mice developed spontaneous colitis and signs of severe chronic inflammation of the gut, probably caused by abnormalities in the epithelial lining of the gut.27 Moreover, double-knockout (abcb1a/b−/−) mice have disturbed renal tubular function caused by decreased intracellular ATP levels and impaired mitochondrial morphology.28 Interestingly, the decrease in proximal tubular reabsorption of amino acids and low molecular weight proteins in these mice was similar to that observed in renal Fanconi syndrome, suggesting the importance of this protein for renal tubular function and not only renal secretion of drugs and metabolites.28

ABCB1 is also involved in the resistance of cells to apoptosis. It has been suggested that this protein may inhibit Fas-induced caspase-8 activation29 and may affect ceramide-induced apoptosis by mediating the translocation of glucosylceramide from the cytosolic to the luminal face of the Golgi.30 Therefore, other than its role in the transport of several drugs and endogenous substrates, ABCB1 may also influence tissue regeneration, because apoptosis contributes to the elimination of damaged cells after injury and tissue remodeling.23

The second protein related to MDR, ABCC1, is expressed at moderate levels in most normal tissues (Figure), ie, polarized epithelia, muscle cells, and also in cells of the immune system.22,31,32 ABCC1 has been implicated in the secretion of organic anions produced by the metabolism of endobiotics and xenobiotics, eg, glutathione-, glucuronide-, and sulfate-conjugated products of phase 2 xenobiotic metabolism.33 The first well-characterized physiological substrate for ABCC1 was leukotriene C4,34 an immune mediator product of activation of the 5-lipoxygenase system for which the function is related to increased cellular permeability and oxidative stress, vascular smooth muscle cell migration, and arterial tone.35 In fact, the main physiological functions attributed to this protein seem to be related to inflammation and oxidative stress via the secretion of leukotriene C4 or the transport of both reduced glutathione and oxidized glutathione.

The third MDR-related protein, ABCG2, is expressed in the apical membrane of several organs, including the kidney and brain. It is highly expressed in the gastrointestinal tract and liver.40–41 (Figure).
be highly influenced by sex steroids and that ABCG2 is highly expressed in the mammary glands during lactation, where it seems to be involved in the secretion of nutrients and dietary carcinogens to milk. Although the physiological role of ABCG2 is at present unknown, it is speculated that ABCG2 may contribute to the pharmacokinetic and pharmacodynamic profiles of several drugs of clinical importance because of their apical location in organs related to drug absorption and excretion (Figure).

Evidence for the Interplay Between ABCB1 and Hypertension: Renal Hemodynamics and Plasma Aldosterone

The first publication concerning the involvement of MDR-related proteins in hypertension was our study showing that a high-sodium diet diminished ABCB1 expression in rats. In addition, a recent study, we showed that ABCB1, but not ABC1, is downregulated in peripheral blood mononuclear cells and in the kidneys of spontaneously hypertensive rats. Our results were confirmed by a study of another group showing that a high-salt diet reduced the expression levels of the abcb1a and abcb1b genes in the rat liver and kidney but increased their expression levels in the duodenum, jejunum, and ileum, suggesting that the regulation of this protein is tissue specific. Corroborating this, it has been shown that ABCB1 undergoes tissue-specific regulation in response to the activation of the nuclear pharnesoid X receptor, because the expression of abcb1a mRNA was induced in the murine small intestine by treatment with the pharnesoid X receptor activators rifampicin and St John’s wort, but its expression in the kidney was not altered.

In addition to the fact that a high-salt diet alters the expression of ABCB1 in rats, we also showed that the mRNA expression of ABCB1 was diminished in adrenalectomized rats, suggesting that aldosterone could be an endogenous regulator of this protein. More recently, it was shown that aldosterone activity in the plasma, brain, and heart was significantly higher in ABCB1-deficient mice. The data obtained in the above studies, performed in different animal models, pointed to a possible role for ABCB1 in hypertension and were followed by additional studies in humans. Aldosterone plays an important role in the pathophysiology of numerous cardiovascular disorders, including hypertension, and the regulation of its plasma disposition and tissue uptake is essential for the maintenance of blood pressure. In 1992, it was first reported that human ABCB1 was able to transport aldosterone, which was confirmed 8 years later by the observation of aldosterone secretion in the human adrenal cell line NCI-H295. Consequently, studies relating ABCB1 expression and its polymorphisms with plasma aldosterone disposition and tissue uptake were undertaken.

A recent study carried on in the Seychelles islands, which are primarily populated by individuals of East African descent, showed that the combined action of ABCB1 and CYP3A5 gene polymorphisms was associated with postproximal tubular sodium reabsorption, plasma renin activity, plasma aldosterone, and an altered blood pressure response to the angiotensin-converting enzyme inhibitor lisinopril. Last year, this same group detected that some ABCB1 gene polymorphisms were related to an altered glomerular filtration rate in caucasians and that other ABCB1 gene variants were associated with renal function and hemodynamic in Africans, suggesting that the ABCB1 gene is related to renal function in the general population. The results obtained by several authors, performed either in animals or in humans, when taken together, strongly suggest that ABCB1 contributes to blood pressure regulation, possibly via the renin-angiotensin-aldosterone system, through the regulation of plasma levels and tissue uptake of aldosterone.

Evidence for the Interplay Between ABCC1 and Hypertension: Hypertensive Response to Angiotensin II

It has been shown that ABCC1 also has a major role in blood pressure regulation. This protein has unusually broad substrate specificity and is capable of transporting a wide variety of neutral, hydrophobic compounds and several glutathione, glucuronide, and sulfate conjugates. Its versatility and ubiquitous tissue distribution allow its involvement in various physiological functions, eg, defense against xenobiotics and endogenous toxic metabolites, leukotriene-mediated inflammatory responses, and protection against oxidative stress.

Endothelial production of reactive oxygen species is increased in hypertension and in oscillatory shear stress, which occurs at points in the circulation predisposed to atherosclerosis. It has been shown that human endothelial cells express ABCC1 and that this protein is responsible for the transport of oxidized glutathione out of these cells. Interestingly, these authors showed that oscillatory shear stress caused a strong export of oxidized glutathione, which was prevented by the ABCC1 inhibitor MK571 and by ABCC1 small-interfering RNA. According to the authors, the inhibition of ABCC1 prevented the decline in intracellular reduced glutathione, preserved the intracellular reduced glutathione Nernst potential, and reduced apoptosis caused by oscillatory shear. Moreover, the altered endothelium-dependent vasodilatation caused by hypertension was ameliorated in ABCC1−/− mice. In a second article, the same group showed that angiotensin II–induced hypertension increased oxidized glutathione export and decreased the vascular levels of reduced glutathione in wild-type but not in ABCC1−/− mice. In this elegant study, the authors showed that aortic endothelium-dependent vasodilatation was reduced in wild-type mice but was unchanged in ABCC1−/− mice after angiotensin II infusion and that superoxide production, expression of several NADPH oxidase subunits, and levels of NO were greatly altered only in wild-type mice. Moreover, the hypertension caused by angiotensin II was markedly blunted in ABCC1−/− mice, strongly suggesting that ABCC1 activity is essential for the hypertensive response to angiotensin II and that this protein might be a new target for the therapeutic handling of hypertension. In a more recent study, this group showed that treatment with MK571 reduced vascular reactive oxygen species production, significantly improved endothelial function, and ameliorated atherosclerotic plaque generation in atherosclerosis-prone, apolipoprotein E–deficient mice on a
Evidence for the Interplay Between ABCG2 and Hypertension: Increased Risk of Stroke and Coronary Artery Disease

The incidence of stroke is continuously increasing in people living in developed countries, and stroke is also emerging as a major problem in developing countries. It is well established that age and hypertension are the most powerful risk factors for stroke.\textsuperscript{66,67} In a very recent cardiovascular health study, in which 74 genetic variants were tested, it was found that a single nucleotide polymorphism of ABCG2 (Val12Met) was associated with increased risk of ischemic stroke in both white and black participants.\textsuperscript{68} The authors showed that homozygotes of the Val allele of ABCG2 were at higher risk of stroke than carriers of the Met allele. This gene variant had been associated previously with angiographically defined severe coronary artery disease in 2 case-control studies.\textsuperscript{69} Because it has been shown that the Met variant of ABCG2 has a lower transport activity and altered pattern of localization when compared with the Val variant,\textsuperscript{70} it was hypothesized that the Met variant of the ABCG2 protein might have a protective function in the vascular endothelium. Interestingly, it has been shown that ABCG2 may have a role in the body regulation of vitamin K3 (also known as menadione), which is necessary for the production of prothrombin and other blood clotting factors in humans.\textsuperscript{71}

As discussed above, evidence obtained recently in 2 studies is consistent with a correlation between the ABCG2 polymorphisms and the leading causes of morbidity and mortality in hypertensive patients, namely, stroke and coronary artery disease. Because so far there is no study relating a direct role of ABCG2 in the genesis of hypertension, more studies relating ABCG2 with stroke and coronary artery disease and its role in vitamin K regulation are necessary to further the meaning of this association.

Treatment of Hypertension: The Role of MDR Proteins in Drug Uptake and Disposition

Other than a role in the genesis and maintenance of hypertension, pharmacokinetic studies have demonstrated the importance of some ABC transporters, eg, ABCB1 and ABCG2, in limiting oral drug uptake and disposition, including several antihypertensive drugs, to target organs. It is long known that interindividual variability in drug response and adverse drug reactions are the main causes of failure in cancer therapy, and the ABC membrane transporters are being now recognized as important determinants of drug disposition, affecting chemosensitivity and drug resistance. In the last few years, several researchers focused on the contribution of genetic polymorphisms of transport proteins to interindividual variability in the efficacy and safety for cancer therapy.\textsuperscript{19,72} However, little is known about the influence of such proteins and their polymorphisms for the effectiveness of antihypertensive therapy.

It has been shown that intestinal ABCB1 expression was increased by spironolactone,\textsuperscript{73} an aldosterone antagonist widely used to treat patients with congestive heart failure, leading to a diminished absorption of digoxin, which is transported by ABCB1 in the intestine and liver.\textsuperscript{74} On the other hand, verapamil, an inhibitor of both ABCB1\textsuperscript{75} and ABCC1,\textsuperscript{76} was shown to increase plasma digoxin concentration attributed to inhibition of ABCB1 activity, resulting in a decreased renal tubular elimination of digoxin.\textsuperscript{77} Also, the toxicity of the interaction between quinidine and digoxin was shown to be attributed to the inhibition of ABCB1.\textsuperscript{78}

Carvedilol, a \(\beta\)-adrenoceptor antagonist with vasodilating activity used for the treatment of hypertension and coronary artery disease,\textsuperscript{79} has been demonstrated to reverse MDR to anticancer drugs in tumor cells in vitro.\textsuperscript{80} This effect is not common to all \(\beta\)-adrenoceptor antagonists, because propranolol, metoprolol, and atenolol (all of them \(\beta_1\)-adrenoceptor selective antagonists) did not affect ABCB1-mediated MDR and transport,\textsuperscript{81} suggesting that this effect of carvedilol was independent of its action on \(\beta\)-adrenoceptors. However, it has been shown that talinolol (a \(\beta_1\)-adrenoceptor selective antagonist) inhibits the ABCB1-mediated transport of digoxin\textsuperscript{82} and that celiprolol (a \(\beta_1\)-adrenoceptor selective antagonist with slight \(\alpha_2\)-agonist action) and acebutolol (a \(\beta_1\)-adrenoceptor selective antagonist) are substrates for ABCB1.\textsuperscript{83–85} More recently, a significant inhibition of polarized, basal-to-apical drug transport of ABCB1 in CaCo-2 monolayers was observed for bisoprolol, carvedilol, and propranolol, whereas metoprolol and sotalol had no effect.\textsuperscript{86,87} Collectively, these data suggest that ABCB1 is one determinant of bisoprolol and carvedilol disposition in humans and that the inhibition of its activity by these drugs possibly contributes to drug interactions, eg, digoxin-carvedilol or cyclosporine-carvedilol, in humans.

Recently, aliskiren, the first drug in a new class of renin inhibitors approved for the treatment of hypertension, was identified as an ABCB1 substrate, which contributes to its relatively low bioavailability.\textsuperscript{8} Both atorvastatin and ketoconazole (known ABCB1 inhibitors), when used at the maximum recommended dose, significantly increased exposure to aliskiren.\textsuperscript{87} However, aliskiren presented no clinically significant drug interaction with digoxin (an ABCB1 substrate).\textsuperscript{87,88} From the pharmacological point of view, those observations suggest that coadministration of aliskiren with other ABCB1 substrates will probably not lead to harmful interactions, but the coadministration of aliskiren with ABCB1 inhibitors should not be recommended.

Several other drugs used in the treatment of hypertension have been proven to be substrates or inhibitors of ABC proteins. For instance, it has been shown that hydrochlorothiazide, a diuretic used worldwide, is a substrate for ABCC4\textsuperscript{89}; the potent diuretic furosemide is transported by ABCC1, ABCC2, and ABCC4\textsuperscript{89,90}; olmesartan, a novel angiotensin II type 1 receptor antagonist, is a substrate for ABCB1, and its prodrug olmesartan medoxomil seems to be a substrate for ABCB1, ABCC2, and ABCG2;\textsuperscript{7} and valsartan, a highly selective angiotensin II type 1 receptor antagonist, is an ABCG2 substrate.\textsuperscript{91}

Conclusions and Perspectives

Although initially discovered in tumor cells, the importance of MDR-related proteins in other diseases is progressively being revealed. Over the past 2 decades, pharmacogenetic
research of human drug-metabolizing enzymes helped us to improve our understanding about the metabolic basis of drug interactions and individual susceptibility to drug toxicity and efficacy. In the last few years, several studies focused on the clinical implications of genetic variations in drug transporters, and this knowledge is guiding the development of new drugs, especially in anticancer research.

The involvement of MDR-related proteins in the genesis, maintenance, and treatment of hypertension is just beginning to be unraveled. The observed interaction between CYP3A5 and ABCB1 genes and urinary sodium excretion in humans suggests a new pathway for blood pressure regulation and has important implications regarding the response to antihypertensive treatment. In addition, the possible altered expression of ABCB1 in hypertension, if confirmed in humans, may have major implications, eg, a possible contribution to the reduced immune function and to altered drug, hormone, and cytokine dispositions in hypertensive individuals, resulting in unusual susceptibility to exogenous or endogenous toxins, as well as to harmful drug interactions.

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Disclosures
None.

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