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Human Organic Transporter 1 (hOCT1) and Response to Imatinib in CML Patients: A Review

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ABSTRACT

Most of the transporters with the ability to translocate organic cations across the plasma membrane belong to the solute carrier family 22A (general gene symbol SLC22A). SLC22A transporter group. It has been well established that the presence of genetic variants in genes encoding proteins involved in drug detoxification processes accounts for inter-individual variability in drug response, and sometimes, this has severe consequences as regards drug toxicity and therapeutic efficacy. OCT1 is also expressed at the basolateral membrane of enterocytes, where, together with the combined transport activity of carriers localized at the apical membrane of these cells, it accounts for the secretion of organic cations toward the intestinal lumen. IM uptake is mediated by the hOCT1 protein encoded by the solute carrier 22 gene (*SLC22A1*). One third of CML patients treated with first line imatinib have suboptimal responses or treatment failures with increased risk for disease progression. This article reviews the role of human organic transporter 1 (hoct1) in eliciting a response to imatinib in cml patients.

Keywords: Human Organic Transporter 1 (hOCT1), Imatinib, CML Patients

INTRODUCTION

Over 350 uptake- or influx transporters have been described, and these are classified into families which broadly reflect their preferred substrates, such as peptides. [1] Imatinib is weakly cationic at physiological pH, and the SLC22 family includes the organic cation transporters, which are known to transport certain drugs.

THE SLC22A1 FAMILY

Most of the transporters with the ability to translocate organic cations across the plasma membrane belong to the solute carrier family 22A (general gene symbol SLC22A).[2] SLC22A transporter group includes 13 well-characterized plasma membrane proteins: 3 organic cation transporters (OCTs), 3 Na^+-zwitterion/ cationcotransporters (OCTNs), and a heterogeneous group of transporters able to transport organic anions (OATs) or urate (URAT). Several members of this family are involved in the uptake of cationic (OCT) and anionic (OAT) drugs across the sinusoidal membrane of hepatocytes. [2]

STRUCTURE OF THE HUMAN ORGANIC TRANSPORTER OCT1

Rat Oct1 was the first organic cation transporter to be cloned. Later, its orthologs were cloned both in humans and mice. [3] The human gene SLC22A1 encoding OCT1 is localized within a cluster on chromosome 6q26 and comprises 11 exons and 10 introns. It consists of 554 amino acids with a predicted membrane topology similar to that of most members of the SLC22A family; that is, it comprises 12-α-helical transmembrane domains (TMD) with N- and C-termini localized intracellularly. [3] There is a large extracellular loop between TMD1 and TMD2, containing glycosylation residues, and a large intracellular loop between TMD6 and TMD7, containing phosphorylation sites. [4]

OCTs consist of many conserved sequence motifs compared to other

members of the major facilitator superfamily, localized between TMD2 and TMD3 and between TMD8 and TMD9. [1] Moreover, an 11-residue sequence found before TMD2 is considered a signature sequence of the OCT family. Moreover, certain cysteine, glycine, and proline residues are conserved in all OCTs cloned to date, suggesting a key role for these residues in establishing the secondary structure of these proteins. Indeed, OCTs show a marked homology between the N- and C-terminal halves, supporting the hypothesis that the structure of these proteins reflects a gene duplication event in the past. [5] On the basis of results obtained from site-directed mutagenesis studies and models of protein tertiary structure prediction of rat Oct1, some amino acids have been suggested to be involved in the substrate translocation pore or in determining the affinity/selectivity for typical cations. [6]

GENETIC VARIANTS and POLYMORPHISMS

It has been well established that the presence of genetic variants in genes encoding proteins involved in drug detoxification processes accounts for interindividual variability in drug response, and sometimes, this has severe consequences as regards drug toxicity and therapeutic efficacy. [7] Genetic polymorphisms in genes encoding drug transporters have also been suggested as a possible mechanism accounting for interindividual variability in drug response by altering pharmacokinetic and hepatic drug clearance. [8] Thus, more than 1000 mutations in theSLC22A1 gene, in the promoter region, in the coding sequence, in the 5′UTR and 3′UTR-regions, or in the introns have been described. However, the biological significance of most single-nucleotide polymorphisms (SNPs) in noncoding regions remains to be elucidated. Regarding the coding sequence of OCT1, the described modifications deposited in the NCBI database include one 3-bp deletion (M420del), 8 nonsense mutations, and 49 missense mutations. Several common nonsynonymous mutations have been found in the SLC22A1 gene in individuals from many ethnic groups, and some of these mutations, such as L160F, P341L, and M408V, have been identified in all of them. These variants, which appear with relatively high frequency, have been reported to maintain transport ability. However, it has also been reported that patients with chronic myeloid leukemia bearing the wild-type genotype GG of the L160F variant show a poorer response to imatinib than patients with the mutation. [9]

Some of the SNPs that result in amino acid substitution severely reduce and alter substrate transport as measured in cellular assays, or may even be of important clinical relevance. Thus, in vitroassays carried out using metformin, MPP^+, or TEA as prototypical substrates, a reduced or even abolished OCT1-mediated transport activity was observed for R61C, C88R, S189L, G220V, P341L, G401S, M420del, G465R, P283L, R287G, P117L, Q97K, R206C, R61S fs*10, and C88A fs*16 . It is striking that several of the variants with reduced activity had altered evolutionarily conserved glycine residues; that is, G220V, G401S, and G465R, suggesting that these residues may be particularly important for OCT1 function. [10]

The expression of OCT1 variants with reduced activity may lead to the accumulation of toxic metabolites and enhanced exposure to environmental toxins, such as the piperidine derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which may reach the brain and be involved in the development of neurodegenerative diseases.

OCT1 variants may contribute to reducing therapeutic drug responses, presumably by decreasing the hepatic uptake of these drugs. Examples are metformin, sorafenib, levodopa, lamivudine, some platinum analogs, tropisetron and ondansetron. A significant association of SLC22A1 mRNA transcription levels with the success of imatinib-based therapy in

leukemia patients has been reported. However, the presence of genetic variants may markedly change the situation. For instance, M420del may alter imatinib pharmacodynamics without changing its systemic pharmacokinetics, resulting in a reduction in the efficacy of this drug in the specific target leukemic cells. Similar results have recently been reported for the use of sorafenib in the treatment of liver cancer. [11]

TISSUE DISTRIBUTION

In humans, OCT1 has a broad tissue distribution, although it is primarily expressed in hepatocytes. In rat hepatocytes, Oct1 has been located at the sinusoidal membrane. OCT1 is also expressed in cholangiocytes. Although to a lesser extent than in rodent kidney, human OCT1 is also expressed at the apical membrane of epithelial cells in the proximal and distal tubules of the nephron. OCT1 is also expressed at the basolateral membrane of enterocytes, where, together with the combined transport activity of carriers localized at the apical membrane of these cells, it accounts for the secretion of organic cations toward the intestinal lumen. [12]

FUNCTIONAL CHARACTERISTICS

The sensitivity of rat Oct1 and human OCT1 the electrical potential across the plasma membrane suggests that they translocate organic cations in an electrogenic manner that is not dependent on Na^+ or H^+ gradients. [13] OCT-mediated transport may occur across the plasma membrane in both directions. OCTs share broad substrate specificity since they can translocate a wide variety of structurally unrelated compounds. Most OCT substrates are organic cations or weak bases that are positively charged at physiological pH, although uncharged compounds may also be transported. Owing to its localization and substrate specificity, the main physiological role of OCT1 is the detoxification of endogenous cationic compounds, but it is also involved in drug disposition. In hepatocytes, OCT1 accounts for the first step in the detoxification of endogenous and xenobiotic compounds, including many drugs, since it carries out uptake across the sinusoidal membrane.

REGULATION OF HOCT1 EXPRESSION AND FUNCTION

Short-term regulation by post-translational mechanisms may result in changes in protein trafficking toward the plasma membrane or in transport affinity, which may occur in response to specific stimuli able to activate phosphorylation or dephosphorylation processes. The results from experiments carried out in two different expression systems- Chinese hamster ovary (CHO) cells and HEK293 cells- have suggested that OCT1 is activated by the Src-like $p56^{lck}$ tyrosine kinase. The Ca^{2+}/calmodulin pathway also stimulates the post-transcriptional regulation of OCT1. Moreover, this study revealed that calmodulin-dependent protein kinase II (CaMKII), a downstream component of this pathway, is involved in OCT1 regulation. The activation of protein kinase C (PKC) decreases the affinity of OCT1 for prototypical substrates. [14]

Regarding long-term regulation, the human OCT1 promoter contains two adjacent putative DNA-response elements for the hepatocyte nuclear factor-4α (HNF-4α). The interaction of HNF-4α with these response elements activates OCT1 transcription, which can be inhibited through the small heterodimer partner (SHP). [15] Since the expression of this transcriptional corepressor can be induced by bile acids, OCT1 expression is reduced in cholestatic liver disease, when elevated bile acid levels counterbalance the HNF-4αmediated activation of OCT1 transcription.

Role of OCT1 in imatinib transport

In chronic myeloid leukemia primary cells and cell lines, imatinib uptake has been shown to be dependent on OCT1. Experiments carried out with OCT1-transfected cells support this hypothesis. The affinity constant of human OCT1 for imatinib has been calculated to be

approximately 5 μM. The degree of OCT1 expression has been suggested to be a useful biomarker to predict the success of imatinib-based therapy in leukemia patients, and, furthermore, leukemia patients who had higher OCT1 expression levels showed a better response to the drug.

DNA HYPERMETHYLATION AND GENE SILENCING

The word "epigenetics" was termed in the early 1940s to describe the events that could not be wholly explained by traditional genetics. The epigenetic field now actively uncovers the molecular mechanisms underlying these phenomena, and epigenetics has been defined today as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence". [16] In other words, epigenetics is the study of changes in gene expression or phenotype, caused by mechanisms other than DNA sequences, and some of these epigenetic changes have even been shown to be heritable. [17]

One of the best studied epigenetic signals is DNA methylation. DNA methylation is a simple covalent chemical modification, resulting in the attachment of a methyl (CH_3) group at the 5' carbon position of the cytosine ring. When methyl groups are attached to the DNA in genes, transcription of these genes is usually turned off, and then these genes are silenced. When a cell divides, its DNA is copied via replication and divided equally into two daughter cells during mitosis. During this process, the pattern of DNA methylation can also be copied onto the new daughter DNA, allowing the information that determines whether a gene is "on" or "off" to be inherited to the two daughter cells. [18]

DNA methylation in mammals occurs mostly at CpGdi nucleotides, and methylation of CpG islands explains a stable gene silencing mechanism. In normal somatic cells, most (over 50%) CpG islands are unmethylated. DNA methylation is important for the regulation of non-CpG islands, CpG island promoters, and repetitive sequences to maintain genome stability. Furthermore, DNA methylation plays important roles in X chromosome inactivation, imprinting, embryonic development, silencing of repetitive elements and germ cell-specific genes, differentiation, and maintenance of pluripotency. [18] DNA methylation is controlled by a family of DNA methyltransferases (DNMTs) that catalyze the transfer of methyl groups from S-adenosyl-L-methionine to the 5' position of cytosine bases in the CpG dinucleotide. Methyl-binding domain (MBD) proteins, such as MeCP2, MBD1, MBD2, and MBD4, bind to methylated CpG sites and are involved in transcriptional repression. DNA hypermethylation has been shown to result in abnormal silencing of several tumor suppressor genes in most types of cancer. Epigenetic silencing via DNA methylation results in gene inactivation and promotes carcinogenesis, indicating that DNA methylation impinges on carcinogenesis.

DISCUSSION

Imatinib mesylate (IM), a well-established gold standard drug in the treatment of chronic myeloid leukaemia (CML), is a synthetic tyrosine kinase inhibitor. Despite excellent efficacy, a significant number of patients on IM therapy develop resistance to IM. Currently, great focus has been laid on the effect of interindividual pharmacogenetic variability on IM treatment responses. IM uptake is mediated by the hOCT1 protein encoded by the solute carrier 22 gene (*SLC22A1*). One third of CML patients treated with first line imatinib have suboptimal responses or treatment failures with increased risk for disease progression.

CONCLUSION

Imatinib is actively transported into cells by the SLC22A1 transporter (hOCT1) and its genetic variants may affect intracellular drug import. The effect of SLC22A1 genetic variants epigenetic

modifications may have a role in modifying long-term outcomes of imatinib treated patients.

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