

Development of High-throughput Human OCT2 Expressing Uptake Assay System



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INTRODUCTION

The kidneys play a key role in the secretion and subsequent elimination of drugs, toxins, and other xenobiotics from the body. Many of these compounds are organic cations in that they carry a net positive charge at physiological pH, including compounds from a broad array of chemical and clinical classes (e.g., antiarrhythmics, β -adrenoreceptor blocking agents, antihistamines, antivirals, and skeletal muscle-relaxing agents). Organic cation transporters in the kidney play essential physiologic and pharmacological roles in the handling of cationic drugs and endogenous organic ions (Koepsell 2007 Pharm Res). Moreover human organic cation transporter 2 (hOCT2) is the most abundant organic cation transporter in the basolateral membranes of renal proximal tubules in human kidney.

Metformin, an antihyperglycemic agent, is mainly excreted into urine, almost entirely in an unchanged form. It is eliminated by tubular secretion in addition to glomerular filtration in the human kidney. Organic cation transporters have been suggested to mediate tubular secretion of metformin; however, the molecular mechanisms underlying the renal tubular secretion of biguanides have not been clarified well. OCT2 was suggested to contribute to the secretion of organic cations, such as tetraethylammonium, metformin, cimetidine, and guanidine.

The goals of this study were to establish a cell culture model which stably expressed OCT2 that could be used to study the characteristics and drug interaction of this transporter, and to study drug-drug interaction in this assay system. In the present study, substrate-transporter interactions were investigated in Chinese hamster ovary cells stably transfected with the human orthologs of the principal organic cation transporter in the kidney, OCT2.

METHODS

To construct the transfectant stably expressing hOCT2, CHO-K1 cells were transfected with the generated pcDNA3.1-hOCT2 DNA plasmid. Four weeks after transfection, single colonies were selected with G418. During selection clones were tested for transport of [14 C]TEA, and the clones that displayed the highest rate of TEA uptake were characterized in greater detail.

Accumulation of [14 C]TEA and [14 C]metformin was measured by the developed high-throughput uptake assay protocol.

CHO-K1/hOCT2 cells were seeded in 96-well plates and the assay was performed in Henseleit-Krebs buffer (HK buffer). The cells were solubilized in 0.5 ml of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. The protein content of the solubilized cells was determined by BCA Protein Assay Kit (PIERCE) with bovine γ -globulin as a standard.

The concentration dependence of TEA and metformin transport by hOCT2 was analyzed using the Michaelis-Menten equation; where V_{max} is the maximum transport rate, $[S]$ is the concentration of TEA or metformin, K_m is the Michaelis constant.

	TEA uptake	Metformin uptake
Cell number	10^5 cells/well	10^5 cells/well
Reaction time	5 min	10 min
pH	8.0	8.0
K_m	71 μ M	170 μ M
V_{max}	400 pmol/mg/min	660 pmol/mg/min
IC ₅₀ Verapamil	7 μ M	2 μ M
IC ₅₀ Quinidine	30 μ M	17 μ M

Table 1. Comparison of parameters obtained by optimized OCT2 mediated TEA and metformin uptake assays.

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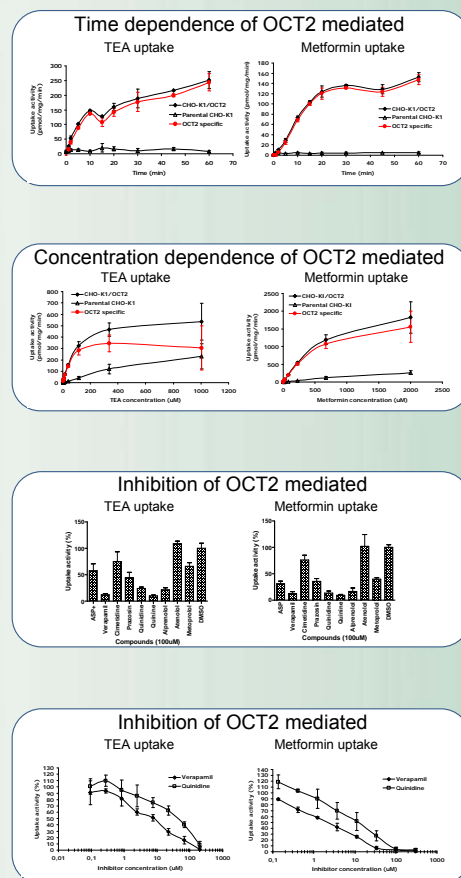


Figure 1: Characterization of the high-throughput OCT2 mediated uptake assay.

RESULTS AND DISCUSSION

We set up a high-throughput cell-based uptake assay system with the selected CHO-K1 monoclonal stably expressing OCT2. Cell culturing and assay protocol was developed on 96-well plate. Cell number and time course of culturing were optimized. Time dependence of the OCT2 mediated tetraethylammonium (TEA) uptake was established (Fig.1). The transport activity was pH-dependent. The uptake of the prototypic type I substrate TEA was saturable and was inhibited by known OCT inhibitors. Values are summarized in Table 1.

Metformin was suggested as a superior substrate for renal OCT2 rather than hepatic OCT1 (Kimura 2005 Drug Metab Pharmacokin), and renal OCT2 plays a dominant role for interaction pharmacokinetics. In order to validate our uptake assay system for a certain pharmacokinetically relevant drug, metformin was also examined using our CHO-K1 cells stably expressing OCT2 (Fig. 1. and Table 1).

The maximum plasma concentration of metformin was reported to be 9–12 μ M after a single oral administration of metformin HCl (850 mg) in patients with type 2 diabetes mellitus and up to 15 μ M and 25 μ M in healthy elderly patients and patients with moderate chronic renal impairment (Sambol 1995 J Clin Pharmacol). Therefore, the transport of metformin by hOCT2 should not saturate at therapeutic concentrations.

Collectively, all the data indicated that the developed high-throughput uptake assay system can serve as a useful and convenient tool in screening candidate drugs for interaction with OCT2 and for studying drug-drug interaction.