論文の内容の要旨

Search and validation of *in vivo* probe drug candidates for the functional analysis of OAT1 and OAT3 in humans

（ヒト*in vivo*で利用可能なOrganic Anion Transporter 1 (OAT1), OAT3の機能評価のためのプローブ候補薬物の探索および検証）

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[Background and Object]

Accumulated clinical evidences indicated that a wide variety of drug transporters as well as metabolic enzymes dominate the pharmacokinetics of drugs in humans, and the expression and function of these molecules are often variable among individuals, resulting in the altered drug concentration and drug response. Generally, the inter-individual difference in the expression and function of these molecules is caused by both genetic factors such as single nucleotide polymorphisms (SNPs) and non-genetic factors such as gender, age, environmental exposure and so on. It is commonly accepted that the functions of metabolic enzymes and transporters still show large inter-individual variation even from the same genetic background. Thus, *in vivo* phenotyping of transporters and enzymes using specific probe substrates or inhibitors for each molecule is one of the powerful approaches to know the quantitative function of pharmacokinetics-related molecules in an individual person. It is also possible to evaluate the quantitative impact of the function of each molecule on the pharmacokinetics of drugs and search for the important molecular targets for drug-drug interaction. For that phenotyping, specific probe substrates and inhibitors for each metabolic enzyme and transporter, which can be clinically applicable to humans, are needed. In the field of CYP enzymes, probe drugs for

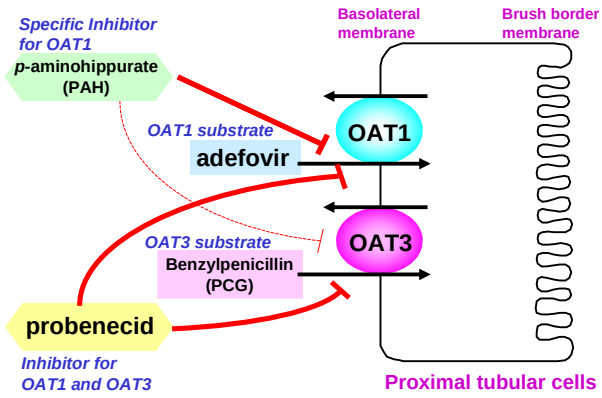


Fig.1 Candidate probe drugs and specific inhibitors for OAT1 and OAT3

each CYP enzyme have been established and simultaneous administration of different probe drugs ("cocktail approach") enables us to quantify the metabolic activity of multiple CYP enzymes at one time. However, as for membrane transporters, though the importance of drug transporter in the drug disposition and elimination in the process of drug discovery and development and the strategies for evaluating the quantitative functions of drug transporters and the extent of drug-drug interaction mediated by transporters have been extensively discussed in the "FDA recent whitepaper on drug transporters" (1), no appropriate method for evaluating the *in vivo* function of multiple transporters important for pharmacokinetics of drugs has established at the moment. Kidney is one of the important organs for the detoxification of xenobiotics including drugs. Recent reports suggested that some membrane transporters are involved in the active tubular secretion and reabsorption processes. Basolateral organic anion transporter (OAT) 1 and OAT3, with broad substrate specificities, are considered to be responsible for the renal uptake of organic anions and the knockout of OAT1 or OAT3 decreased the renal clearance of some drugs, such as furosemide and benzylpenicillin in mice. Therefore, the purpose of my study is to establish and validate the appropriate candidates of human *in vivo* probe drugs and specific inhibitors for OAT1 and OAT3 by integrating the results of *in vitro* study and human clinical drug interaction study.

[Methods and Results]

As candidates of probe drugs for renal uptake transporters in humans, adefovir and benzylpenicillin (PCG) were selected as specific substrates for OAT1 and OAT3, respectively. Moreover, *p*-aminohippurate (PAH) was used as a specific inhibitor for OAT1 and probenecid as a potent inhibitor for both OAT1 and OAT3 (Fig. 1). The substrate specificities of probe substrates (adefovir and PCG) and the inhibitory effects of PAH and probenecid on their uptake were evaluated in transporter expression systems and human kidney slices. Furthermore, a human clinical drug interaction study was performed to observe the effects of the coadministration of PAH and probenecid on the pharmacokinetics of adefovir and PCG in healthy volunteers.

1. <u>The specific recognition of candidates of probe substrates and inhibitors in *in vitro* studies</u>

Adefovir and PCG are specifically taken up into OAT1- and OAT3-expressing HEK293 cells, respectively, suggesting that these compounds can be used as specific substrates of OAT1 and OAT3. Probenecid can potently inhibit the uptake of both adefovir and PCG with the K_i values of 12.6 and 3.00 μM in human kidney slices, respectively (Fig. 2). In contrast, PAH preferentially inhibited OAT1-mediated adefovir uptake (18.6 μM, Fig. 2A) rather than OAT3-mediated PCG uptake (499 μM, Fig. 2B), indicating that an appropriate concentration of PAH can be used as a specific inhibitor for OAT1 in the clinical situation.

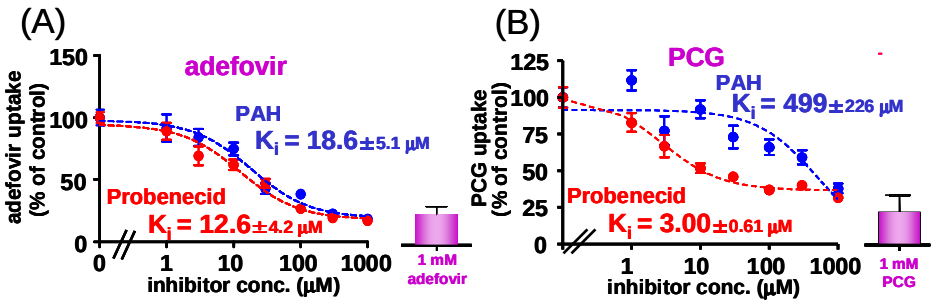


Fig. 2 Inhibitory effects of PAH and probenecid on the renal uptake of adefovir (A) and PCG (B) in human kidney slices

2. <u>Pharmacokinetics of adefovir and PCG in Oat1 and Oat3 knockout mice</u>

An *in vivo* study using Oat1 and Oat3 knockout mice was carried out to compare the renal clearance of adefovir and PCG in wild type and knockout mice. After intravenous administration of adefovir in Oat1 knockout mice, its plasma concentration was significantly increased and renal clearance was decreased to half compared with wild type mice. By subtracting glomerular filtration clearance from renal clearance, its secretion clearance was estimated to be almost nothing in Oat1 knockout mice. These results indicated that Oat1 plays an important role in the tubular secretion of adefovir. Mr. Kodaira in our laboratory previously demonstrated that the total clearance of PCG was decreased to 40.5% and its renal secretion clearance also decreased, even lower than the glomerular filtration clearance in Oat3 knockout mice compared with wild type mice. From these results, Oat3 largely contributes to the renal secretion of PCG and significant reabsorption mechanism of PCG existed in Oat3 knockout mice.

Therefore, it is suggested that Oat1 and Oat3 are important to determine the *in vivo* renal clearance of adefovir and PCG, respectively, which supports our *in vitro* results.

3. <u>Clinical drug interaction study between probe drugs for OAT1 and OAT3 (adefovir and PCG) and inhibitors (PAH and probenecid) in healthy volunteers</u>

This study protocol was approved by the ethics committees of both the faculty of pharmaceutical sciences in the University of Tokyo and Kitasato University East Hospital. 24 healthy male volunteers were randomly divided into four groups and 6 subjects in each group were treated with one of 4 combinations of substrates (adefovir or PCG) and inhibitors (PAH or probenecid). Three different doses of inhibitors (PAH or probenecid) were coadministered with probe substrates (adefovir or PCG) to each subject. The renal clearance of adefovir was decreased (50～83 % and 48～71 % of control) by both probenecid and PAH in a dose-dependent manner, respectively as we expected from the results of *in vitro* inhibition assays. In the case of PCG as a substrate, its renal clearance was decreased (24～53 % of control) by probenecid, but contrary to our expectation, coadministration of PAH increased the renal clearance of PCG. This phenomenon may be explained by the inhibition of reabsorption process of PCG.

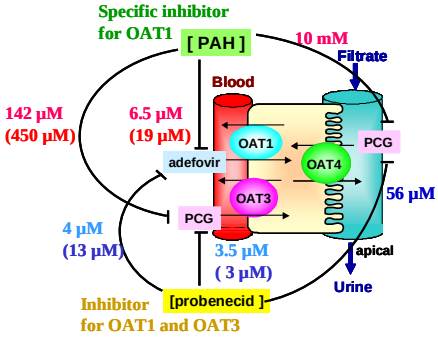
4. <u>Possible mechanism for the tubular reabsorption of PCG</u>

Up to now, although not well characterized, some apical membrane transporters expressed in renal tubular epithelial cells are considered to be involved in the renal reabsorption of compounds. To further characterize the underlined mechanism of the inhibitory effect of PAH on the reabsorption of PCG, *in vitro* uptake study of PCG in gene expression systems was performed. The significant uptake of PCG could also be observed in human OAT4-expressing MDCK cells and inhibited by both probenecid and PAH with K_i values of 56 μM and 11 mM, respectively. In the clinical study, at the

high dose of inhibitors, the average unbound plasma concentration of probenecid and PAH was 56 μM and 827 μM, respectively. At present, there is no good method to accurately estimate the real concentration of inhibitor at the vicinity of transporters. If the reabsorption of PCG mainly occurs in the proximal tubule, and assuming that urine concentration of drugs at the proximal tubule is estimated to be 3.3-fold higher than their plasma concentration according to the literature information, the maximum concentration of PAH in the primitive urine was estimated to be about 2.7 mM in phase 4, which is smaller than the K_i value for OAT4 (11 mM). From this point, PAH could not strongly inhibit OAT4 function under the condition of my clinical study and we cannot conclude whether OAT4 partly contributes to the reabsorption of PCG *in vivo*. Another candidate transporter for reabsorption, URAT1 did not transport PCG into its expression system in a normal condition, suggesting that URAT1 is not involved in the reabsorption of PCG.

[Discussion and Conclusion]

Taking all the results into consideration, as we expected from the *in vitro* study, adefovir can be used as a probe drug for estimating the *in vivo* function of OAT1. PCG can be used as a probe drug for OAT3 only in its renal basolateral uptake process, while in our clinical drug interaction study between PAH and PCG, PAH increased renal clearance of PCG due to the possible inhibition of its reabsorption. So it was suggested that the renal clearance of PCG was affected not only by OAT3 but also by undefined reabsorption mechanisms. Thus, a special attention for reabsorption should be taken to consider the pharmacokinetics of PCG. It is also important to further clarify the exact mechanisms of renal reabsorption of drugs and to establish the methods for quantitative prediction of the impact of reabsorption on the overall renal clearance, which will help us the appropriate selection of OAT3 probe drugs. Previous reports indicated that large inter-individual variability of mRNA expression of OAT1 and OAT3 was observed in humans, though the frequencies of their genetic polymorphisms are very rare. Thus, using probe drugs, we expect that the inter-individual variation of the *in vivo* transport function of OAT1 and OAT3 could be estimated just by measuring the blood and urine concentration of probe substrates. Also, in the process of drug development, probe substrates have a great potential to be a useful tool to help the detection of clinically important drug-drug interactions in humans.



The data represent Ki values from OAT1,3-expression HEK 293 cells(without parenthesis) and kidney slices(with parentesis), respectively.

Fig. 3 Summary for the effects of inhibitors (PAH and probenecid) on the uptake of probe substrates (adefovir and PCG)

[Acknowledgement] I am grateful to Gilead Science Inc. for their kindly providing us with adefovir. I would also like to thank Dr. Tsunenori Kondo (Tokyo Women's Medical University) for the provision of human kidney slices for our studies and Dr. Yuji Kumagai (Kitasato University East Hospital) with his medical staffs and volunteers for the great contribution to our clinical study.

[References]

(1) Giacomini, K. M. et al. (2010) Membrane transporters in drug development *Nat Rev Drug Discov* **9**(3), 215-36.