論文の内容の要旨

論文題目：Functional analysis of MRP4 on the urinary excretion of cephalosporin antibiotics

(セフェム系抗生物質の尿細管排出における Multidrug Resistance-Associated Protein 4 (MRP4/ABCC4)の関与)

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[Introduction]

Cephalosporin antibiotics are one of the most important groups of antibiotics. They have a cephem nucleus with various side chains at the 3- and 7- positions of the β-lactam and dihydrothiaizine ring, respectively, and are classified into four generations based on their general features of antimicrobial activities. Most cephalosporins are excreted into the urine in unchanged form, while a few cephalosporins, such as cefoperazone, cefpiramide and cefodizime are excreted predominantly into the bile. According to the population pharmacokinetic analysis, it was found that the renal clearance of ceftizoxime exhibited a bimodal distribution due to an unknown mechanism. Genetic factors have been hypothesized to account for this bimodal distribution.

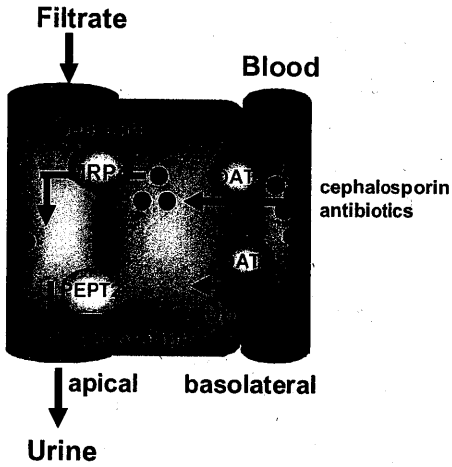

Fig. 1 The mechanism of renal excretion

The urinary excretion of drugs and their metabolites consists of glomerular filtration and tubular secretion. Tubular secretion of drugs has been characterized by organic anion and cation transport systems. Cephalosporins are known to be substrates of basolateral organic anion transporters (OAT1 and OAT3). Since OAT3 exhibits greater transport activity than OAT1, OAT3 has been considered to play a major role in the renal uptake process. There are a few single nucleotide polymorphisms affecting the functional activity of OAT1 and OAT3, however, their allele frequencies are too rare to account for the bimodal distribution. Therefore, the present study focused on the subsequent efflux process, particularly focusing on multidrug resistance-associated protein 4 (MRP4/*ABCC4*), a member of the ABCC family which expressed in the brush border membrane of the proximal tubules in the kidney. This study investigated whether MRP4 is involved in the tubular secretion of cephalosporins, and whether the polymorphisms of MRP4 relate to the various pharmacokinetic parameters of cephalosporins.

[Methods and Results]

1. Transport of cephalosporins by MRP4

Human MRP4 and mouse Mrp4 were expressed in an HEK 293 cell line using adenovirus expression systems. The effect of various cephalosporins on the ATP-dependent uptake of [^{3}H] dehydroepiandrosterone sulfate by MRP4-expressing membrane vesicles was examined. The results showed that most of the cephalosporins are inhibitors of MRP4, and injected cephalosporins were more potent inhibitors than oral ones with the exception of cefepime, cefsulodin and cefaloridin, which showed no effect on MRP4 mediated transport of [^{3}H]DHEAS. The ATP-dependent transport of cephalosporins by MRP4 was examined in the vesicular study. Time- and ATP-dependent transport of ceftizoxime, cefazolin, cefotaxime and cefmetazole were observed (Fig 2). The K_m and V_{max} values for the ATP-dependent uptake of ceftizoxime by MRP4 were 18.3 ± 2.2 mM, and 529 ± 26 pmol/min/mg protein and, for cefazolin, the corresponding parameters were 80.9 ± 10.9 mM and 3.24 ± 0.25 nmol/min/mg protein.

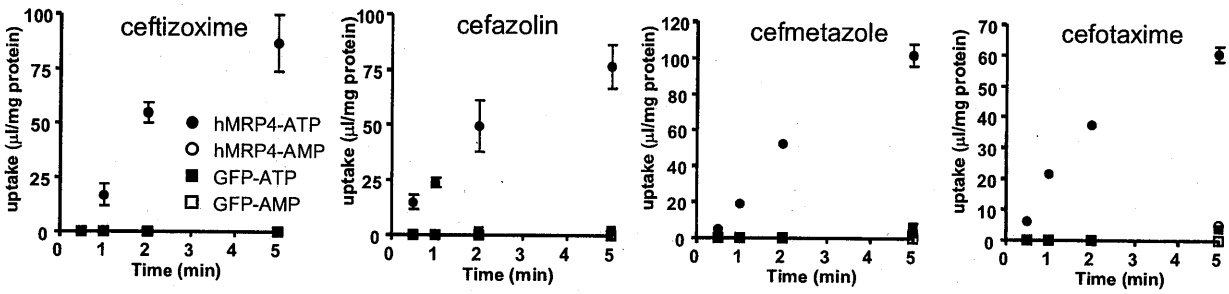

Fig. 2 Time-profiles of the uptake of various cephalosporins by human MRP4-expressing vesicles.

2. Renal excretion of ceftizoxime, cefazolin in Mrp4 (-/-) and wild-type mice

An in vivo study using Mrp4 knockout mice was carried out to determine the renal clearance of ceftizoxime and cefazolin between the wild-type and Mrp4 knockout mice (Fig 3). The plasma concentrations after intravenous infusion of ceftizoxime were similar in Mrp4 (-/-) mice and wild-type mice, while they were slightly higher in Mrp4 (-/-) mice than wild-type mice for cefazolin. The urinary excretion of ceftizoxime was significantly reduced in Mrp4 (-/-) mice compared with wild-type mice, while there was no significant difference in the urinary excretion rate and the total clearance of cefazolin between Mrp4 (-/-) mice and wild-type mice. The kidney accumulation of ceftizoxime and cefazolin was significantly higher in Mrp4 (-/-) mice than wild type mice (1.9- and 3.4-fold, respectively), while the renal clearance with regard to the kidney concentration were reduced in Mrp4 (-/-) mice (51 and 37% of the control, respectively). The in vivo results indicate that Mrp4 makes a significant contribution to the tubular secretion of cephalosporins.

3. Functional analysis of SNPs variants of MRP4

Four single nucleotide polymorphisms (SNPs) of MRP4 encoding for amino acid changes (G171C, G187W, K304N and E757K) were generated using site-directed mutagenesis, the expression levels and

transport activities were determined using the membrane vesicles from HEK 293 cells infected with the recombinant adenoviruses containing cDNA of the MRP4 variants. All variants exhibited lower protein expression, particularly the expression level of G171C was 50% of the control. Transport activity of ceftizxime by MRP4 was lower in G171C MRP4 variant (~50%) than those in other variants due to lower protein expression, while K304N and E757K MRP4 variants exhibit greater transport activities of dehydroepiandrosterone sulfate (140% of wild-type). For *p*-aminohippurate, G187W MRP4 variant exhibited greater transport activity (140%). These results suggest that the effect of SNPs is substrate-dependent, and G187W variation is associated with decreased renal clearance of ceftizoxime due to lower protein expression level.

4. Uptake of ceftizoxime by kidney slices

The renal uptake of ceftizoxime was investigated using kidney slices. Saturable uptake of ceftizoxime was observed in kidney slices with a K_m value of 3.78 ± 0.95 mM. The inhibitory effect of substrates of OATs, such as PAH and benzylpenicillin, and a non-specific inhibitor of organic anion transporters, probenecid, on the uptake of ceftizoxime by kidney slices was examined. The inhibitory effect of *p*-aminohippurate and benzylpenicillin was lower than that of probenecid, sufficient to saturate their own uptake. Namely, it is likely that other transporters distinct from OAT1 and OAT3 are responsible for the uptake of ceftizoxime.

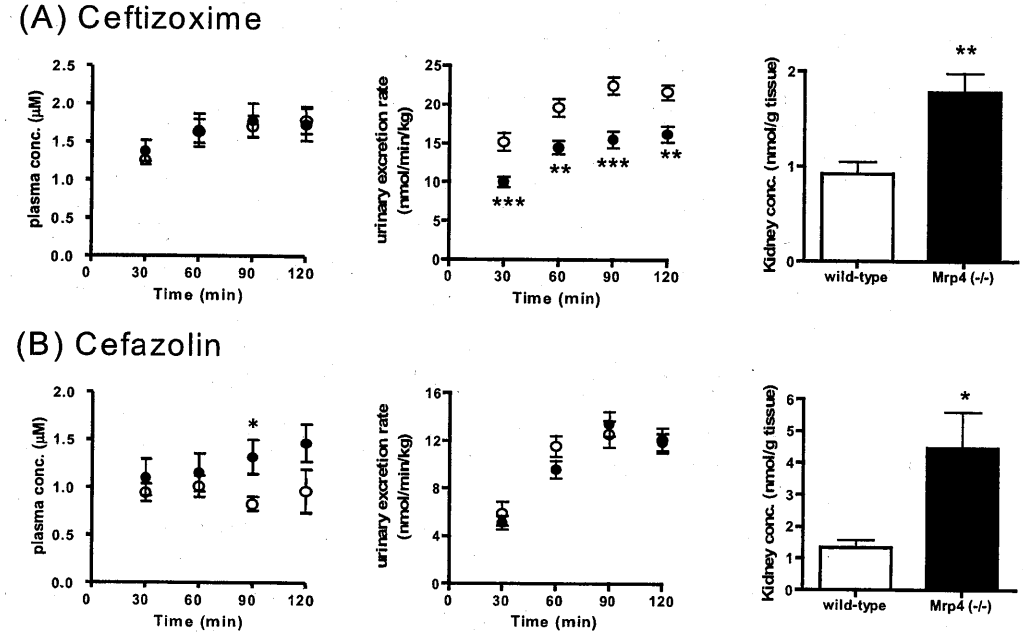

Fig. 3 Time profiles of the plasma concentration, urinary excretion and kidney concentration of ceftizoxime, cefazolin in Mrp4 (-/-) and wild-type mice.

[Conclusion and discussion]

The present study suggests that some cephalosporins are substrates of MRP4, and it accounts for the luminal efflux of ceftizoxime and cefazolin together with an unknown transporter. The effect of SNPs of

MRP4 is likely to be substrate dependent. G187W is expected with decreased renal clearance of ceftizoxime due to lower protein expression level. An uptake study using kidney slices suggests the involvement of an unknown transporter in the renal uptake process. Genetic factors of this transporter may account for the bimodal distribution.

[Acknowledgement]

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