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Drugs interacting with organic anion transporter-1 affect uptake of Tc-99m-mercaptoacetyl-triglycine (MAG3) in the human kidney: Therapeutic drug interaction in Tc-99m-MAG3 diagnosis of renal function and possible application of Tc-99m-MAG3 for drug development

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ABSTRACT

Introduction: Renal uptake of Tc-99m-MG3 involves organic anion transporter (OAT). Treatment with drugs showing OAT affinity might interfere with renal uptake of Tc-99m-MAG3, leading to misinterpretation in Tc-99m-MAG3. This study was conducted to discuss a possible drug interference with Tc-99m-MAG3 diagnosis on OAT sites.
Methods: Renal uptake and plasma clearance of Tc-99m-MAG3 were analyzed in healthy volunteers under control and OAT1 and OAT3 related drug treatment conditions. An in vitro uptake study using OAT1 or OAT3 expressing cells was also conducted.
Results: Both PAH and probenecid treatment induced delays in Tc-99m-MAG3 clearance from blood, and reductions in the renal uptake clearance. As a result, the normalized effective renal plasma flow estimated from Tc-99m-MAG3 clearance was significantly underestimated, whereas the glomerular filtration rate estimated from plasma creatinine levels was unchanged. The transport activity of Tc-99m-MAG3 was higher in OAT1-expressing cells than in OAT3-expressing cells.
Conclusion: Drugs with OAT1 affinity affect the renal uptake of Tc-99m-MAG3 and blood clearance. This might cause misinterpretation of functional diagnosis of the kidney using Tc-99m-MAG3.

1. Introduction

Although much has been published on renal transporters and drug interactions [1], in vivo imaging using a radiopharmaceutical is an underdeveloped area [2]. Tc-99m-mercaptoacetyl-triglycine (Tc-99m-MAG3) was introduced in 1986 [3], and has become the radiopharmaceutical of choice in the evaluation of transplant kidneys, diagnosis of tubular necrosis, and scintigraphic study of tubular function. The image quality when using Tc-99m-MAG3 is superior to that with I-131-orthoiodohippurate (OIH) [4], and is adequate for monitoring renal function [5].

The urinary excretion of compounds is determined by glomerular filtration, tubular secretion and reabsorption from the urine. Glomerular filtration is determined by the unbound fraction in the plasma and GFR, whereas transporters play an indispensable role in the ability of the epithelial cells, forming proximal tubules, to remove the compounds from the blood. Complementary DNA (cDNA) encoding a *p*-aminohippurate transporter designated as Organic Anion Transporter-1 (OAT1)/*SLC22A6* has been isolated [6]. OAT1 is expressed in the middle segment of the proximal tubule in the kidney, and acts as a multispecific transporter mediating the uptake of various drugs, such as diuretics, beta-lactam antibiotics, and antiviral agents [7,8]. It has been demonstrated that Tc-99m-MAG3 is a substrate of OAT1 in cDNA-transfected cells, suggesting that OAT1 accounts for the kidney uptake of Tc-99m-MAG3 from blood [9]. In addition to OAT1, OAT3/*SLC22A8*, an OAT1 isoform, is also predominantly expressed in the basolateral membrane of kidney proximal tubules [10]. Because OAT3 shows overlapped substrate specificity with OAT1, it is also possible that OAT3 is involved in the renal uptake of Tc-99m-MAG3 together with OAT1. Thus, drug treatment might cause an alteration of the renal uptake and excretion pattern of Tc-99m-MAG3 by inhibiting the

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transporters, potentially causing misinterpretation of renal function diagnosis based on Tc-99m-MAG3 clearance.

In the present work, a Tc-99m-MAG3 study was performed in healthy volunteers under control and drug-treated conditions in a crossover fashion, and the effect of drug treatment was evaluated by ordinary diagnostic procedures as well as pharmacokinetic analysis. "Sodium *p*-aminohippurate injection (10%)" and "benecid tab 250mg (probenecid)" were selected as a relatively selective OAT1 substrate [6,10,11] and a potent OAT1 and OAT3 inhibitor [12–14], respectively. The former is approved as a drug for diagnosis of kidney function, and the latter approved for the treatment of hyperuricemia as well as prolongation of the effectiveness of penicillin antibiotics drug. Thus, a single dose of these drugs does not affect the physiological status of the kidney. Probenecid has been known to inhibit the tubular secretion of various anionic drugs at its clinical doses [8,15]. Comparison of the unbound concentration in the plasma and inhibition constants of probenecid indicates that inhibition of the uptake process by OAT1 and OAT3 is in vivo relevant at its therapeutic dose [15].

Based on the results obtained, the possible interference of drug treatment in functional diagnosis of kidneys using Tc-99m-MAG3 and the possible contribution of OAT1 and/or OAT3 to the renal uptake of Tc-99m-MAG3 in human were discussed.

2. Materials and methods

2.1. Materials

Tc-99m-MAG3 Injection was purchased from Fujifilm RI Pharma Co., Ltd. (Tokyo, Japan). Creatinine was purchased from Sigma Chemical Co. (St. Louis, MO), and PAH and probenecid were purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals used were commercially available and of analytical grade.

2.2. Clinical studies

2.2.1. Subjects

This study was approved by the Institutional Review Board and informed consent was obtained. Twelve healthy male volunteers (age range 22–25 y; mean age 22.9± :1.1 y) were included in this prospective study. At entry, the mean serum creatinine level of all the subjects was 0.84 ± 0.12 mg/dl (range 0.66–0.99). Subjects were not allowed to take any medications, foods which include Saint John's wort, or fruit juice (grapefruit, orange, or apple) for seven days before the study.

2.2.2. Pre-treatment procedure

All the subjects were scanned twice with Tc-99m-MAG3 on two separate days. On the first day of the study they were scanned without any pretreatment (control scan). Between 7 and 29 (mean 10.0 ± 6.5) days after the first scan, they were scanned with a pretreatment as described below (drug-loaded scan). The subjects were separated into two groups; the probenecid group and the PAH group. The subjects in the probenecid group took 750 mg of probenecid orally one hour before Tc-99m-MAG3 injection. In the PAH group, intravenous infusion of 120 mg/min of PAH was started before scanning.

2.2.3. Control scan protocol

Technetium-99m-Mercaptoacetyl-triglycine (Tc-99m-MAG3, 200 MBq/mL, 0.050 mg/mL as benzoyl-MAG3) was purchased from Fujifilm RI Pharma Co. Ltd., Tokyo, Japan. The scanning protocol followed instructions for commercially available e-cam signature software (e-soft Version 3.5.7.13_SP6 SR3.1) based on the method previously reported [16]. Thirty minutes after oral intake of 250 ml water, the subject was placed in the supine position on the imaging table. They received an intravenous injection of 300 MBq of Tc-99m-MAG3, and posterior dynamic imaging was performed for 60 min using a gamma camera (E.CAM, Toshiba-Siemens, IL, USA). After Tc-99m-MAG3 injection, serial 1-second/frame digital images were obtained for 60 seconds, followed by 30 10-second/frame images and 53 60-second/frame images for a total study duration of 60 min, and data were processed. Time zero was defined as the point of Tc-99m-MAG3 injection. One subject could not tolerate delaying urination until the end of the study, and the study was ended after 40 min. The injection syringe was also imaged to estimate the injected count. (A hollow paper box 20 cm in height was put on the imaging table above the detector head. The syringe was placed on the paper box before and after injection, and data were acquired for 30 sec each.)

For further detailed analysis, venous blood samples were also obtained from the arm contralateral to the injection site before and 1, 3, 5, 10, 30 and 60 min after injection of Tc-99m-MAG3. Three milliliters of blood was used for radioactivity measurement and 5 ml for drug/creatinine concentration measurement. The blood samples were centrifuged to separate the plasma. Activity concentration in the plasma was evaluated by a gamma counter (1480 Wizard 3" Automatic Gamma Counter, Perkin Elmer, Inc., Waltham, MA, USA). The radioactivity concentration was decay-corrected to the time of injection, and the results were expressed as the radioactivity per 1 g plasma.

2.2.4. Drug-loaded scan protocol

For the probenecid group, 1 hour after taking 750 mg of probenecid, Tc-99m-MAG3 was injected, and then dynamic scanning was performed for another one hour. For the PAH group, after 10 min of PAH administration at 120 mg/min, PAH injection was stopped and Tc-99m-MAG3 was injected, then PAH injection resumed and was continued during scanning. The dosage regimens of probenecid and PAH were decided based on the clinical study we conducted in healthy volunteers. Detail of the study was presented in 23th Annual Meeting of JSSX (Kumamoto, Japan) in 2008 (https://www.jstage.jst.go.jp/article/jssxmeeting/23/0/23_0_178/_pdf). For both group, venous blood samples were obtained before and 1, 3, 5, 10, 30 and 60 min after Tc-99m-MAG3 injection.

2.2.5. Quantification of plasma creatinine, PAH and probenecid in the plasma specimens

The plasma specimens were deproteinized with 10-fold volumes of acetonitrile, and centrifuged at 20,000 × g for 10 min. The supernatants were then diluted with 100-fold volume of mobile phase and subjected for LC–MS analysis. The plasma concentrations of creatinine were measured by LC–MS/MS as described previously [17]. Estimated glomerular filtration rate (eGFR) was obtained from the plasma creatinine level as follows:

$$\text{eGFR (mL/min/1.73 m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287}$$ [18]

(Cr: plasma creatinine (mg/dL), Age: year)

$$\text{eGFR(mL/min/kg)} = \text{eGFR(mL/min/1,73 m}^2) \times \text{BSA/1.73}$$

$$\text{(BSA(body surface area)} = \text{Weight(kg)}^{0.425} \times \text{height(cm)}^{0.725} \times 7184 \times 10^{-6}\text{)}$$ [19]

An LCMS2010EV equipped with a Prominence LC system (Shimadzu, Kyoto, Japan) was used for the analysis of PAH and probenecid. Chromatographic separation was achieved on a phosphorylcholine hydrophilic interaction chromatography column (3 μm, 2.0 mm × 150 mm; Shiseido, Tokyo, Japan) under isocratic conditions for PAH and a Capcell Pak C18 MGII column (3 μm, 2.0 mm × 50 mm; Shiseido, Tokyo, Japan) in binary gradient mode at a flow rate of 0.4 mL/min for probenecid; 10 mM ammonium formate and acetonitrile (15:85, v/v) for PAH and 0.1% formic acid and acetonitrile for probenecid were used for the mobile phase, respectively. The acetonitrile concentration for probenecid was