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Endogenous metabolites that are substrates of organic anion transporter's (OATs) predict methotrexate clearance

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ABSTRACT

Variable pharmacokinetics of high-dose-methotrexate (MTX) is responsible for severe toxicities. Unpredictable overexposure still occurs during some courses despite having controlled the main factors known to play a role in its elimination. The aim of our study was to evaluate whether the urine metabolomic profile measured at the time of MTX administration is predictive of the drug's clearance and/or of treatment-related toxicity. We analyzed the urine content of endogenous metabolites before MTX administration in a cohort of adult patients treated for lymphoid malignancies. Individual MTX clearance (MTX_{CL}) was estimated from population pharmacokinetic analyses of therapeutic drug monitoring data. We determined the urine metabolite content by gas chromatography-mass spectrometry (GC–MS) and applied Partial Least Square (PLS) analysis to assess the relationship between the urine metabolome and MTX_{CL}. External validation was applied to evaluate the performances of the PLS model. We used orthogonal partial least squares discriminant analysis (OPLS-DA) to distinguish patients with normal or delayed elimination, and patients with or without toxicity. Sixty-two patients were studied. We obtained a very good prediction of individual MTX clearance using a set of 28 metabolites present in patient urine at baseline. The mean prediction error and precision were −0.36% and 21.4%, respectively, for patients not included in the model. The model included a set of endogenous organic anions, of which the tubular secretion depends on organic anion transporter (OAT) function. Our analyses did not allow us to discriminate between patients with or without delayed elimination or those who did or did not experience toxicity. Urinary metabolomics can be informative about an individual's ability to clear MTX. More broadly, it paves the way for the development of a biomarker of tubular secretion, easily measurable from endogenous substances.

Abbreviations: MTX, methotrexate; HD-MTX, high-dose methotrexate; OH-MTX, hydroxymethotrexate; UCP (I/I + III) ratio, urinary coproporphyrin ratio; MRP2, multidrug resistance related protein-2; TDM, therapeutic drug monitoring; POP-PK, population pharmacokinetics; H24, 24 h after the start of MTX infusion; Hx, x h after the start of MTX infusion; CTCAE, common terminology criteria for adverse events v.4.03; NONMEM, nonlinear mixed effect modeling program; GC–MS, gas chromatography–mass spectrum; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; EI, electronic impact; QC, quality control; PCA, principal component analysis; UV, unit variance; DModX, distance to the model plane; MTX_{CL}, clearance of methotrexate; VIP, variable importance on projection; CV-ANOVA, ANOVA of the cross-validated residuals; RMSE-CV, root mean square error from cross-validation; RMSE-P, root mean square error of prediction; ME, mean error; PE, prediction error; RMSE, root mean squares error; OPLS-DA, orthogonal projections to latent structures discriminant analysis; ROC, receiver operating characteristic; OATs, organic anion transporters.

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1. Introduction

Large inter- and intra-patient variability of systemic exposure is observed after administration of high-dose methotrexate (HD-MTX). These variable pharmacokinetics result in very high MTX concentrations in some patients. This can cause potentially severe unpredictable toxicity, necessitating aggressive resuscitation leading to prolonged hospitalization and sometimes death. The main mechanism of MTX elimination after intravenous infusion is renal excretion, with biliary secretion contributing less than 20% [1,2]. Renal elimination of MTX and its major metabolite, 7-OH-MTX, proceeds by glomerular filtration and active secretion involving membrane transporters of proximal tubular cells [3]. A number of factors that predispose patients to altered MTX pharmacokinetics have already been identified. Genetic polymorphisms of enzymes or transporters involved in MTX elimination are associated with variable MTX clearance [4–12]. However, these are host-specific factors that do not vary and should lead to similar serum concentrations from one course to another, which is not the case. Course-to-course variability may arise from variable renal function, drug-drug interactions, or other environmental factors, but the exact cause is not always identified [13–15]. Overexposure still occurs during some courses despite controlling all factors known to alter MTX pharmacokinetics before prescription, even in patients for whom previous courses were well-tolerated. Thus, there is a need for a new biomarker which can reflect an individual's capacity to clear the drug from the body for each separate administration of the drug. We previously studied the urinary content of coproporphyrins I and III to measure the coproporphyrin ratio (UCP (I/I + III) ratio) as a potential biomarker for the activity of the tubular transporter MRP2 [16]. We found a relation between MTX clearance and variations in coproporphyrin levels during the course of treatment. However, these metabolites were not predictive of MTX clearance, probably because they reflect a unique pathway.

In the current study, our aim was to analyze in depth the global metabolite content of urine, because it may elucidate the contribution of all the processes controlling MTX urinary elimination, without the assumption of the predominant role of any one mechanism. Metabolomics has been used as a source of predictive biomarkers to optimize patient management in numerous studies [17–19]. It consists of analyzing all low-molecular weight compounds present in a biological sample to generate metabolite profiles that may better describe a patho-physiological state. The ultimate aim is to correlate these metabolic profiles to any observed phenotype. Here, we used an untargeted gas-chromatography-mass spectrometry platform to explore the urinary metabolome of patients treated with HD-MTX to determine whether basal, pre-treatment metabolic profiles could predict MTX clearance (CL_{MTX}). The urinary metabolomic profiles were obtained from patients of a prospective population pharmacokinetic (POP-PK) study of HD-MTX in adults with lymphoid malignancy. We also evaluated whether the basal urinary metabolite profile could predict delayed elimination or severe toxicity of MTX.

2. Patients and methods

2.1. Recruitment of patients

The prospective COMETH study (clinicaltrial.gov number NCT00822432) was conducted in patients hospitalized in the hematology department of the University Hospital of Tours, France and in the hematology and neurology departments of Pitié-Salpêtrière University Hospital in Paris, France. All patients were more than 18 years of age and were prescribed chemotherapy with high-dose (HD)-MTX (>1 g/m^2) for a lymphoid malignancy. Patients who had received MTX within the three months before enrollment were not included. The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The COMETH protocol was approved by the ethics committee of Pitié-Salpêtrière University Hospital and all subjects gave written informed consent for the study.

2.2. Study protocol

Patients were monitored during the first HD-MTX infusion, administered over three hours. Pre-hydration and urine alkalinization with intravenous sodium bicarbonate to maintain urine pH > 7 were applied.

Therapeutic drug monitoring of MTX is routinely performed to determine whether patients are at high risk for MTX toxicity, and to guide folinic acid rescue therapy using nomograms based on the serum concentrations of MTX at various times post-infusion. Supplemental injections of folinic acid are provided if delayed elimination is observed, and until the methotrexate concentration falls below a given threshold, generally set at 0.1 or 0.2 μM [20]. Discharge is permitted only if the serum MTX level is below this threshold. This threshold is 0.2 μM in our hospital.

Therapeutic drug monitoring samples were collected at H24 (24 h after the start of MTX infusion), H48, H72, and then every 24 h until concentrations fell below the non-toxic threshold of 0.2 μmol/L. An additional blood sample was collected at the end of the infusion (H3).

The concentration measured at H72 was used to assign patients to the "good elimination" group (H72 < 0.2 μM) or "delayed elimination" group (H72 ≥ 0.2 μM).

Urine samples were collected over the 24 h preceding the start of alkaline pre-hydration before the HD-MTX course and stored at −20 °C until metabolomic analysis.

2.3. Assessment of toxicity

Biological variables (blood cell counts, serum creatinine, liver enzymes) and clinical parameters reflecting muco-cutaneous, digestive or other toxicities were recorded on Day 1 and then once a week after MTX administration. Toxicities were graduated according to recommendations of the Common Terminology Criteria for Adverse Events v.4.03 (CTCAE).

2.4. Determination of methotrexate clearance

Individual methotrexate clearances were obtained through a population pharmacokinetic analysis using the nonlinear mixed effect modeling program NONMEM (version VI; Globomax LLC, Hanover, USA). Details of the modeling process have been described previously [16]. Data were fitted by a two-compartment model with exponential and proportional error models for inter-individual and residual variabilities, respectively. Three covariates explained inter-individual variability of MTX clearance (MTX_{CL}), *i.e.* creatinine clearance, the variation of the UCP I/(I + III) ratio over the course, and the presence or absence of at least one drug known to interfere with MTX elimination. Individual MTX_{CL} were obtained using the Posthoc function of NONMEM.

2.5. Urine metabolomic profiling

Gas chromatography–mass spectrum (GC–MS) analyses were conducted as described by Emond et al. [21]. Briefly, each sample was thawed at room temperature and the creatinine concentration measured to adjust the volume of sample to a creatinine concentration of 1 mM. The urine was then extracted using a liquid-liquid procedure and, after solvent evaporation, derivatized using