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A systematic toxicity evaluation of cephalosporins via transcriptomics in zebrafish and *in silico* ADMET studies

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ABSTRACT

Cephalosporins are beta-lactam antibiotics that are widely used in clinics in China. However, information on their toxicity to zebrafish is limited. This study reports that the toxicity effects of cephalosporins containing an N-methyltetrazolthio ring at the C-3 position (CNMTs) exposure on zebrafish were comparable to those predicted by in silico analysis. The effects of CNMTs on the mortality and malformation rate of zebrafish were concentration-dependent. The transcriptional levels of the has1 and cnnm2a genes, which are related to embryo development and absorption of $Mg2+$ in vivo, significantly changed. Several pathways that were enriched by differentially expressed genes (DEGs) were identified, and the most significantly co-enriched pathways were related to neuroactive ligand-receptor interactions, cardiac muscle contraction, and vascular smooth muscle contraction. In sum, the C-3 substituent in the nucleus 7-aminocephalosporanic acid (7-ACA) of CNMTs is responsible for the observed toxicity at higher concentrations, and the C-7 substituent plays an important role in toxicity at lower concentrations. Our results show that zebrafish embryos and transcriptomics may be useful for determining target organ toxicity, assessing the structure and toxicity relationship of chemicals, and improving drug safety assessments.

1. Introduction

Cephalosporins are classic examples of chemical modifications of an original natural product, cephalosporin C. Cephalosporins are characterized by their nucleus, 7-aminocephalosporanic acid (7-ACA), which is (semi-)synthesized by modifying the substituents at the C-3 and C-7 positions. Most of the second- and third-generation cephalosporins contain an *N*-methyltetrazolthio ring at the C-3 position (CNMTs) such as cefoperazone (CFP), cefminox (CMN), cefmetazole (CMZ), cefmenoxime (CMX), cefotiam (CTM), latamoxef (MOX), and cefotetan (CTT) (Fig. 1). In particular, MOX has been modified to have an oxygen atom in the cephem ring instead of a sulfur atom. These have strong antibacterial ability, a wide antimicrobial spectrum, and minimal adverse reactions. Their common side effects include allergic reaction, renal injury, and blood and hematopoietic system, digestive system, and nervous system damage (Bhattacharyya et al., 2016). Several studies have indicated that the *N*-methyltetrazolthio (MTT) ring at the C-3 position is responsible for hypoprothrombinemia and hemorrhage (vitamin K deficiency) and Antabuse-like effect (inhibition of aldehyde dehydrogenase), and the carboxylic group at the C-7 side chain can cause platelet dysfunction (Katukuri et al., 2016). To date, several cephalosporin-related safety issues remain unresolved, mainly because of the lack of understanding of the mechanism underlying toxicity. It is thus essential to elucidate the mechanism of toxicity, define the genes or proteins responsible for the toxicity, and determine biomarkers to screen for the drug toxicity.

Pharmacokinetic (PK) properties of cephalosporins are mainly based on their chemical structure, which affects their bioavailability, absorption, distribution, degradation and elimination, and even toxicity. Toxicological characterization of cephalosporins in mammalian remain the gold standard for toxicity prediction in humans, but they are expensive, time-consuming, require large amounts of test compounds and experimental facilities. Furthermore, *in silico* toxicology methods can help to cut down time and costs. The computational models that predict the relationships between chemical structure and toxicological effects has been applied in regulatory toxicology (Alqahtani, 2017; Kleandrova et al., 2015; Merlot, 2010). In addition, such computational models can be used not only at the level of molecular targets, but also at different levels of biological complexity such as cells, microorganism, tissues, laboratory animals, and even humans (Speck-Planche and Cordeiro,

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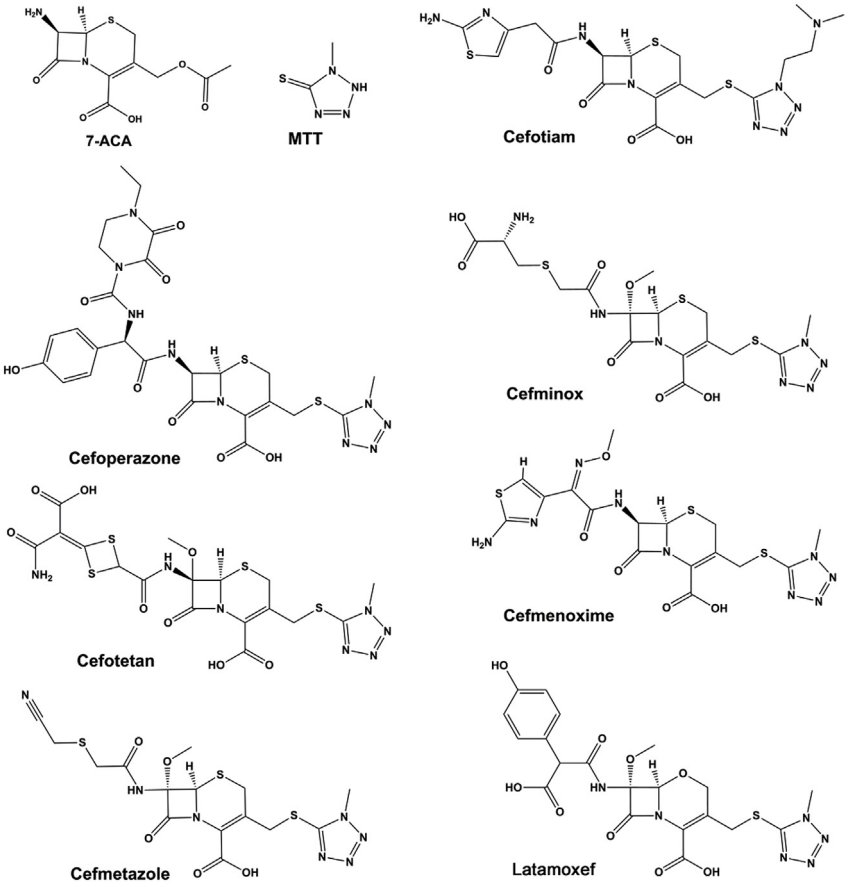

Fig. 1. Chemical structures, common names, or abbreviations of nine compounds investigated in this study.

2014a, 2014b).

Currently, zebrafish has become an important alternative animal model in toxicology and drug safety evaluation (Cornet et al., 2017; García-Caballero et al., 2018; Yoganantharjah and Gibert, 2017). Abundant drug toxicity screening have been conducted using zebrafish embryos (Dale et al., 2017; Wang et al., 2016). Our group has also established the zebrafish embryo toxicity models to assess the safety of chemicals and drugs (Chen et al., 2017; Han et al., 2015, 2017, 2018). Our previous study showed that the C-3 and C-7 side chains are toxic functional groups of cephalosporins (Zhang et al., 2013), MTT at the C-3 position of CFP may induce bleeding in zebrafish (Han et al., 2017), and the C-3 substituent group MMTD (2-mercapto-5-methyl-1,3,4-thiadiazole) is responsible for cardiac toxicity of cefazolin in zebrafish (Chen et al., 2017).

To improve the efficacy and safety of drugs in patients and to reduce adverse drug reactions due to exposure to cephalosporins, this article aimed to expand current knowledge on the interactions involving cephalosporin PK, toxicity, and related mechanisms, predict PK using *in silico* models, and reveal the relationship between toxicity and structure of CNMTs in zebrafish.

2. Materials and methods

2.1. Laboratory animals

All experiments were approved by the Committee on the Ethics of Animal Experiments of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences (Beijing, China, IMBF20060302), which is according to the NIH Guidelines for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/index.htm). AB wild-type zebrafish were maintained under a 14-h light/10-h dark cycle in an automatic circulating tank system. The freshwater temperature was maintained at 28 ± 1 °C and pH 7 ± 0.5. Embryos used for chemical exposure were obtained from pairs of spawning adult zebrafish, which were placed in a breeding tank. Fertilized embryos were selected for all experiments as previously described (Han et al., 2017). The developmental stages of the embryos and larvae were expressed in hours post-fertilization (hpf) or days post-fertilization (dpf).

2.2. Chemicals

The reference standards for cefoperazone (CFP, CAS No. 62893-19-0), cefminox (CMN, CAS No. 75481-73-1), cefmetazole (CMZ, CAS No. 56796-20-4), cefmenoxime (CMX, CAS No. 65085-01-0), cefotiam (CTM, CAS No. 61622-34-2), latamoxef (MOX, CAS No. 64952-97-2), cefotetan (CTT, CAS No. 69712-56-7), 7-aminocephalosporanic acid (7-ACA, CAS No. 957-68-6), and 5-mercapto-1-methyltetrazole (MTT, CAS No. 13183-79-4) were obtained from the National Institutes for Food and Drug Control (Beijing, China). The structure of each compound was confirmed by MS and NMR; the purity of compound (> 95%) was normalized by HPLC or by NMR.

2.3. Prediction of ADMET by computational analysis

PK properties such as absorption, distribution, metabolism, and excretion (ADME) and toxicity profiling of compounds were determined using an ADMET descriptors algorithm protocol of pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and Discover Studio 4.0 (DS 4.0) software package (Accelrys Software Inc., CA, USA). Some important chemical descriptors correlate well with PK properties, such as the topological polar surface area (TPSA, a primary determinant of fractional absorption) and the octanol–water partition coefficient