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Photocatalysis as a method used in drug metabolism simulation *in vitro*

Introduction

The introduction of the new molecular entity (NME) to the pharmaceutical market is an immensely expensive, time-consuming and complex process. It requires a lot of efforts made by qualified specialists from various fields of science and generates huge costs at every single step of the research as well. The approval of new substance in order to use in medicine is preceded by years of preclinical development and multi-stage clinical trials. The complexity of these processes directly translates into the possibility of the appearance of expensive mistakes. This, in fact, underlies the focus of specialists in establishing a universal protocols allowing or excluding from subsequent stages of NME development so as to slash this risk. The preclinical drug metabolism evaluation is supposed to hold a particular value in drug development program. It has been noticed that inauspicious pharmacokinetic parameters of the substance, as well as the formation of unfavorable, reactive or toxic metabolites may result in severe health consequences for patients after drug administration. Taking this above into account, the possible drug withdrawal from market due to the regulatory oversight decision could be the foundation of huge financial losses of the pharmaceutical company.

The medicinal substances are subjected to well-known metabolism principles. The biotransformation process aims at changing the chemical character of a molecule to more hydrophilic in order to facilitate its further elimination in urine or faeces. The metabolism is a biochemical process divided into two phases. First of them consists of various redox reactions like heteroatom oxidation, delkilation, hydroxylation and hydrolysis, which results in hydrophilic moieties introduction to the compound structure. Intermediates formed during this step are eliminated as such or undergo specific coupling with endogenic cofactors i.e.

glucuronic or sulphuric acid concurrently becoming a participant of Phase II. The liver is considered to be the organ of human body predominantly involved in drug metabolism due to the highest levels of enzymes responsible for biotransformation processes. That discovery has become the reason for establishing of a medicinal substances metabolism evaluation protocol based on the liver model. Henceforth, the incubation with human liver microsomes (HLM) is the frequently used assay for this purpose.

The understanding of the oxidoreductive nature of biochemical reactions associated with the metabolism of medicinal substances drew the researchers attention to the possibility of using the heterogeneous photocatalysis process in regard to the biotransformation studies. The reaction mechanism is based on the created reactive oxygen species (ROS) demonstrating strong oxidizing potential and their capability of interacting with organic compound molecules. Since it has been proven that the course of photocatalytic oxidizing reactions are similar to biotransformation processes, the photocatalysis became novel method in drug metabolism simulation studies.

Objectives

The main goal of this study was to perform the metabolism simulation experiments of chosen medicinal substances with the use of photocatalytic method. The model titanium dioxide nanoparticles, as well as the catalysts with yet undocumented efficiency in this type of research were selected to fulfill this assumption. Furthermore, in order to select the most efficient catalyst, a comparison of twelve metal oxides was used upon a set of twenty model pharmaceuticals with a diverse chemical structure. The next aim was to perform the structural elucidation of detected metabolites based on the recorded high-quality spectra in the course of carried out HR-LC-MS analyzes. In selected cases, additional *in silico* toxicity evaluations were made for the previously identified metabolites. The next objective of this research was to study

the impact of the catalyst particle size on the metabolism simulation efficiency. Additionally, the feasibility of photocatalytic method use in metabolite production and further isolation has become the final aim of this research.

Materials and methods

The studies were conducted using twenty three selected medicinal substances as a pure standards or isolated from pharmaceutical formulation. The photocatalytic experiments were mainly conducted in a Suntest CPS + photostability test chamber, equipped with a xenon burner and D65 filter, providing irradiation in the wavelength range 290 – 800 nm. In the experiment with citalopram, the Dymax BlueWave® 200 UV Light-Curing Spot Lamp was used as the radiation source. Fifteen photocatalysts with nanoparticle structure were used throughout this research. Maintaining the proper stiring of suspensions was ensured by the use of magnetic stirrer. The HLM incubations were carried out in a shaken water bath at a thermostated temperature of 37 °C or in an incubator allowing temperature control and mixing (Eppendorf).

The samples obtained in both experiments were subjected to quantitative and qualitative analysis with the use of system consisting of the hybrid high resolution Q-TOF mass spectrometer coupled with ultra-high performance liquid chromatograph with DAD detection (Agilent). A positive electrospray (ESI) ionization mode was used as the ion source. All the chromatographic experiments were conducted in the reverse phase (RP) mode, with the use of C-18 column (Phenomenex). Water containing 0.1% of formic acid and acetonitrile were used as a mobile phase. The isolation of the main molindone metabolite was performed using ultrafast liquid chromatography (UFLC) system (Shimadzu) and semi-preparative (C-18) chromatographic column (Agilent). The collected fractions containing the investigated biotransformation product were evaporated using a rotary evaporator (Heidolph), dried under a stream of nitrogen, and then subjected to ^{1}H NMR analysis (Bruker).

The computational evaluation of the selected biotransformation products toxicity was performed using the following software: ACD/Percepta (ACD/Labs), Vega, T.E.S.T. and Ecosar. The obtained results were submitted to the multivariate chemometric analysis with the use of Mass Profiler Professional (Agilent) and R software (GNU Project).

Results and discussion

During this research the biotransformation of nine selected drug substances was studied with the use of photocatalytic approach and incubation with the HLM as a biological reference method. The chemometric analysis of registered metabolic profiles performed with the use of Mass Profiler Professional software, indicated the preferred shorter irradiation time as a one of the photocatalytic method parameters. The number of single experiments carried out as well as the performed multivariate chemometric comparison of activity among selected twelve metal oxides highlighted the high efficiency of tungsten (VI) oxide and zinc oxide in the context of drug metabolism simulation studies.

Based on the tungsten (VI) oxide, selected in this way, the impact of photocatalyst nanoparticle size on the course of metabolic processes was investigated as well. The comparison using chemometric data analysis techniques showed a clear preference for the molecules subjected to greater micronization.

The process of metabolites production for their subsequent isolation is a procedure that facilitates accurate structural determinations and further analysis. Therefore, the possibility of producing metabolites using the photocatalytic method was investigated. The obtained promising results allowed considering the application of the proposed solution as a cheaper, faster and requiring less specialized equipment compared to the methods used so far.

The UHPLC-Q-TOF MS combined system was used for the sample analyzes and recorded high resolution spectra which were used to structurally identify many new

biotransformation products. Additionally, computational *in silico* toxicity assessment was performed for selected identified metabolites. Due to the varied chemical structure of the metabolites and the use of several models of different versions of the software, it is not possible to determine one leading trend as to the acute toxicity of metabolism products. On the other hand, the prediction of genotoxic activity indicated a high probability of its occurrence against the epoxidized flunarizine derivative, as well as high receptor toxicity for the identified, not yet reported in the literature, hydroxylated metabolite of citalopram. Both metabolites stood out in the terms of expected toxic effects against a number of computationally evaluated biotransformation products.

Conclusions

Taking into account the outcomes of the study, the heterogenic photocatalysis was found to be a valuable preliminary method of drug metabolism simulation *in vitro*. The proposed solution benefits from simple procedure and relatively low cost. Its complementary use with other assays results in obtaining a wide range of date related to the course of biotransformation processes of medicinal substance. Furthermore, the ability to produce significant quantity of metabolites simplifies accurate structural elucidation procedure due to easier selection of high quality LC-MS spectra. This is also the basis for considering the photocatalytic method as an efficient approach of obtaining metabolites for further isolation and performing detailed analyzes. On the basis of the identified structures, the *in silico* toxicity assessment could be performed, which provides basic knowledge about the potential risk of using the particular medicine. Another asset of photocatalytic simulation method is the possibility to mimic extrahepatic biotransformation mechanisms which improves its versatility in this type of research.

Despite the same mechanism of action, there are explicit differences between photocatalysts in regard to their efficiency. Performed chemometric analyzes pointed out the WO_3- and ZnO-assisted photocatalysis to be the best approach in drug metabolism simulation studies. It was noticeable, that their efficiency outperformed model titanium dioxide nanoparticles. The course of photocatalytic simulation process could be subjected to some improvements, and the use of smaller catalyst particle should be considered as the one of them since it has been proven that it ensures better similarity of metabolic profile.