

## Diclofenac: Exploring the microbial degradation pathway

Joanna zur\*, Agnieszka Nowak, Justyna Michalska, Danuta Wojcieszynska and Urszula Guzik

\* University of Silesia in Katowice, Poland; E-mail: jozur@us.edu.pl

### Abstract

A wide range of unique chemicals of emerging concern (CECs), including pharmaceuticals, are continuously introduced into natural environment including water and soil matrices, mainly from hospital and municipal wastewater or manufactures. Currently, diclofenac (DCF; [2-(2,6-dichloroanilino)phenyl] acetic acid), classified as nonsteroidal anti-inflammatory drug (NSAID) constitutes one of the most serious problem worldwide. Due to its frequent occurrence in wastewaters and natural waters diclofenac is even proposed as a suitable marker for anthropogenic pollution, which confirmed the great importance of NSAIDs environmental pollution. Moreover, as a consequence of its environmental significance, diclofenac is currently classified in the watch list, which contains the most important candidates for a supplemented list of priority substances for the WFD (European Water Framework Directive). Up to now, only a few bacterial strains able to DCF decomposition have been described. Moreover, so far only a few of the initial metabolites of the microbial degradation of diclofenac (including hydroxylated and lactam derivatives) have been identified. Structural and metabolic changes occurring in the bacterial cells under the influence of this drug also remain poorly characterized. The main aim of this research was to describe the microbial degradation pathway of DCF in *Pseudomonas* strains. The analysis include high performance liquid chromatography, gas chromatography coupled with mass spectrometry, measurement of specific enzymes activity presumably involved in diclofenac degradation e.g. hydroquinone 1,2-dioxygenase, hydroxyquinol 1,2-dioxygenase, catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, protocatechuate 3,4-dioxygenase and protocatechuate 4,5-dioxygenase. The influence of diclofenac on bacteria was determined by analysis of the composition and content of fatty acids (FAME, fatty acid methyl esters), which build the bacterial membrane. Toxicity of DCF was evaluated by calculation of EC50 value.

Diclofenac, sold under the brand name Voltaren among others, is a nonsteroidal antiinflammatory drug (NSAID) used to treat pain and inflammatory diseases such as gout. It is taken orally, rectally in a suppository, used by injection, or applied to the skin. Pain improvements last up to eight hours. It is also available in combination with misoprostol for the purpose of reducing stomach problems. Common side effects include abdominal pain, gastrointestinal bleeding, nausea, dizziness, headache, and swelling. Serious side effects can include heart disease, stroke, kidney problems, and stomach ulceration. Use n ' is not recommended in the third trimester of pregnancy. It is probably safe while breastfeeding. It is thought to work by decreasing the production of prostaglandin. It blocks both

cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Diclofenac was patented in 1965 by Ciba-Geigy; it entered medical use in the United States in 1988. It is available as a generic drug In 2017, it was the 94th most commonly prescribed drug in the United States, with more than eight million prescriptions. It is available as sodium and potassium salt.

Microbial biodegradation is the use of bioremediation and biotransformation methods to harness the natural ability of microbial xenobiotic metabolism to degrade, transform, or accumulate environmental pollutants, including hydrocarbons (e.g. petroleum), polychlorinated biphenyls (PCBs) , polyaromatic hydrocarbons (PAHs), heterocyclic compounds (such as pyridine or quinoline), pharmaceuticals, radionuclides and metals. Interest in the microbial biodegradation of pollutants has intensified in recent years, and recent major methodological advances have enabled high-throughput genomic, metagenomic, proteomic, bioinformatic and other analyzes of environmentally relevant microorganisms, providing new information on biodegradation pathways and the capacity of organisms. to adapt to changing environmental conditions. Biological processes play a major role in the removal of contaminants and take advantage of the catabolic versatility of microorganisms to degrade or convert these compounds. In environmental microbiology, global genomebased studies improve understanding of metabolic and regulatory networks, The study of the fate of persistent organic chemicals in the environment has revealed an important reservoir of enzymatic reactions with a high potential for preparative organic synthesis, which has already been exploited for a certain number of oxygenases on a pilot and even industrial scale. . New catalysts can be obtained from metagenomic libraries and from approaches based on DNA sequences. Our increasing capacity to adapt catalysts to specific reactions and process requirements by rational and random mutagenesis widens the field of application in the fine chemicals industry, but also in the field of biodegradation. In many cases, these catalysts need to be exploited in whole cell bioconversions or in fermentations, which requires system-wide approaches to understand strain physiology and metabolism and rational approaches to whole cell engineering, as they are increasingly offered in the field of systems. biotechnology and synthetic biology The consumption of diclofenac was associated with a significant increase in vascular and coronary risk in a study including coxib, diclofenac, ibuprofen and naproxen. Upper gastrointestinal complications have also been reported. Major cardiovascular events (MACE) were increased by about a third by diclofenac, mainly due to an increase in

major coronary events. Compared to placebo, out of 1,000 patients allocated to diclofenac for one year, three more had major vascular events, one of which was fatal. Vascular death was significantly increased by diclofenac.

#### Biography

Joanna Żur has studied the microbial degradation pathways of nonsteroidal anti-inflammatory drugs since the beginning of her PhD studies, realized in the Department of Biochemistry, Faculty of Biology and Environmental Protection, University of

Silesia in Katowice under the supervision of Urszula Guzik. The second main direction in her work is immobilization of whole bacterial cells. Recently, our research group described the microbial degradation pathway of paracetamol, the most important analgesic drug worldwide. Up to now, the most important scientific achievements in her career is the authorship and co-authorship of review and research articles from the Philadelphia list of journals. Besides this, she worked as the Leader or Executor in eight scientific grants, including national and international projects. She is a Member of Polish Society of Microbiologists.