

MOLECULAR BIOLOGY TECHNIQUES & CELL AND ORGANOTYPIC CULTURES IN ASSESSMENT OF PHARMACOLOGICAL CHARACTERISTICS OF STANDARDIZED FRACTIONS OF BEE PRODUCTS

R. STOJKO¹, Zofia DZIERŻEWICZ³, J. STOJKO², A. STOJKO² Agata PYTEL¹

¹Department of Pathology, Faculty of Pharmacy, Medical University of Silesia, Katowice, POLAND, E-mail: rstojko@slam.katowice.pl

²Department of Sanitation, Bioanalysis & Environment Research, Faculty of Pharmacy, Medical University of Silesia, Katowice, POLAND

³Department of Molecular Biology, Biochemistry and Biopharmacy, Faculty of Pharmacy, Medical University of Silesia, Katowice, POLAND

Introduction

The development of the drug science is determined by accomplishments of basic sciences as well as medicine and technology. These fields of human thought have enabled and brought about the pharmacodynamic research necessary in assessment and standardization of pharmacologically active substances, originally acquired from materials of biogenic origin. Observations made on the present conditions proved a starting point for acquiring synthetic drugs. The parallel development of biotechnology, pharmacology and phytochemistry has led to a considerable progress in the scope of detection methods, isolation and analysis of substances such as glycosides, alkaloids and flavonoids. These discoveries were accompanied by deeper and deeper reaching pharmacodynamic studies characterizing a drug's biological accessibility, establishing its pharmacological and toxicological characteristics. The development in basic sciences - especially in molecular biology and genetics - has contributed to recognize different mechanisms accompanying the pharmacodynamic and pharmacokinetic processes disclosing depending on the kind of drug, its form and conditioning of an organism, these being a result of interaction between numerous genetic and non-genetic factors. Pharmacogenetics provides us with a number of instances where genetic and environmental factors co-exist and co-work.

Studies show that each human race has a different reaction to some active substances present in drugs. It is the Caucasian and Asian races that show the biggest differences. It is also known, that a sick person within a single race show different reactions to a drug depending on his age, sex, general health as well as other taken drugs. There are proofs showing that genes can influence the efficacy and efficiency of different methods of pharmacological treatment. This is caused by the fact that mainly genes are responsible for the synthesis of enzymes taking part in chemical reactions occurring in our bodies, including the direction and pace of chemical changes resulting from prescribed drugs. These findings are partly the result of observations of patients with tuberculosis who were treated with isoniazid. In some patients the unmetabolized drug remained in the blood for a longer period of time. It was those patients which suffered from some neurological disorders; others did not experience them. Genetic studies made on these patients, which differently reacting to the therapy, showed two different types of inherited isoniazid metabolism - the so-called slow and fast ones.

In the recent years, different forms of genes have been discovered, which protein products take part in the process of chemical changes as enzymes. Doctors usually have no doubts about type and dose of a drug when there are ailments caused by a single gene. The process of mapping of the single nucleotide polymorphism (SNP) present in the entire genome is being continued. A single nucleotide change observed in the sequences of genes coding proteins which transport and metabolize drugs is the basis of pharmacogenomics. However, when there is more than one gene playing an important role in a particular disease, choosing the right therapy is not simple. Unfortunately, the multi-gene diseases constitute the potentially most numerous group of all diseases. The so-called "hot-spots" are being currently looked for; these are the DNA fragments which, because of the differences they show in various humans - are responsible for different reactions of an organism to a particular drug. Pharmacogenomics - a new field in biomedicine - will eventually treat each patient individually, based on his or her SNPs set or "hot-spots" in DNA molecules.

Pharmacogenomics is a field which will be responsible for "target treatment" and which will be a part of the next revolution in medicine, argues, in a famous scientific magazine, Francis COLLINS, President of the American National Human Genome Research Institute. This new branch of medicine will allow us not only to save time and money often wasted on inefficient treatment, but will also minimize the side effects of pharmaceuticals. The pharmacoepidemiological data clearly shows the undesirable effects of numerous registered pharmaceuticals. Reports published in the US indicate that every one out of four people dies there because of drugs' harmful effects. Disturbing data is coming from e.g. France, saying that every fourth drug allowed for sale there has been found either ineffective or even dangerous to one's health or life. It is also believed that pharmacology will enable to master the process of acquiring new medicines. In order to develop a new drug, it is necessary to know the 3D structure of proteins, enzymes, nucleic acids and cell surface receptors. Pharmaceutical companies are already collecting samples of human DNA, hoping that in

this way they will be able to create a profile enabling them to classify the patients and divide them into those the drug will help, and those it will harm. The development of a new drug is a long-term process, starting with basic studies done in laboratories and multi-phase clinical testing used to evaluate drug's effectiveness and harmlessness as a potential method of treating a particular disease. Before a drug is tested on humans, its safety is proved during animal and cell culture tests. In some cases of products collected, changed or produced by bees, clinical studies have proved their positive role in the treatment of particular diseases.

The constant progress made in biomedical studies – being the consequence of the rapid development of molecular biology – brings forth new experimental possibilities for gaining knowledge about the mechanisms of influencing a human organism by different single chemical substances – as well as their complex systems – deriving directly from nature, e.g. insect communities, among which we find the surely exciting example of bees' colonies.

Propolis is one of the pharmacopealic materials used in apitherapy, of course, apart from honey. This biogenic product's bactericidal properties have been known for a long time. Currently, it is believed that the bactericidal properties of propolis are the product of the synergic effect of flavonoids, aromatic acids and sesquiterpens. Its auxiliary effect in treatment of all kind of burns, bedsores, varicose vein ulceration and eczemas has been shown in clinical studies. Spectacular results obtained from it in treating so many illnesses have also shown that apart from its bactericidal properties, propolis also helps to regenerate the damaged tissues. Studies done on a cellular model proved that the standardized propolis extract increased the prolific activity of fibroblasts. And the molecular studies revealed that propolis is responsible for an increased transcriptive activity of genes taking part in the angiogenesis process^[1]. The confrontation of clinical studies with basic studies has enabled us to explain the mechanisms of its activity on the cellular and molecular level. Also, getting to know the cellular and molecular mechanisms of the active fraction of bee products in the physiological and pathological systems fills in the gaps and helps their position not just in prevention but in treatment as well.

A number of facts support the idea that the short-chain fatty acids (SCFA) created in the human large intestine play numerous and important physiological roles. It is believed that disturbances in their metabolism may be one of the causes of infectious diseases of the large intestine. From the energetic point of view, out of all fatty acids, it is the butyric acid that is most desired for the mucous membrane. It has been shown that a decrease of its concentration in the lumen of the colon – being the consequence of a lower inflow of substrates easily being fermented – leads to atrophy of the mucous membrane of this particular part of the digestive tract. Supplying extra butyrate in the form of inlets regenerates the mucous membrane, which is shown by the intensification of its epithelium growth as well as the deepening of intestine's crypts^[2]. It is being postulated that butyrate may be an important factor in protection against the large intestine cancer since it has been shown in the *in vitro* studies that it has a disciplinary influence on the cancer cells^[3]. At the moment, its natural sources are being looked for. Our attention is focused on bee bread because the latest literature^[4, 5] indicates that 14% of its entire content consists of organic acids. In our Biopharmacy Institute (Molecular Biology, Biochemistry and Biopharmacy Department, Silesian Medical University), we have developed a quantitative method of determining SCFA in bee bread^[4, 5]. In the analyzed bee bread, we noticed the presence of different SCFAs, including the butyric acid. We also showed that the process of drying them has a negative effect on their SCFA content.

It seems that an important step ahead in the studies of bee products will be made when there will be the possibility to utilize the primary cultures as well as different kinds of cell lines deriving from a human organism in order to analyze the influence of these raw and standardized preparations on morphological and biochemical parameters of cells used in the cultures. It is no doubt a good alternative to studies on animals, which, however, do not always show us the processes taking place within human organs or tissues.

New techniques of culturing cells and human tissues *in vitro* have been developed in the last 20 years. The possibility of obtaining a mass culture from a small segment, the progress in finding out about the biology of many types of cells as well as the ability to modify their actions in cultures have all led to the growth of interest for the practical use of these achievements not only in biology, but in medicine as well. It has been noted that cells deriving from a healthy connective tissue are easy to grow. Methods have been developed to culture - out of epithelial cells – cells of skin keratinocytes, urinary tract epithelias, prostatic gland, oral cavity, vagina and eye cornea. The epidermis is an especially good tissue to culture because over 90% of it falls into one category of cells – keratinocytes. Keratinocytes – in appropriate conditions – are stable in culturing, do not lose their ability to proliferate and differentiate. The main reason for failures in grafting the epidermis is the lack of its attachment to the wound, e.g. after burns, which probably results from the lack of proper skin in the graft. This problem, however, has been overcome by developing the living skin equivalent (LSE). It has been shown that the human skin acquired from culturing bears numerous similarities to the natural skin in the morphological and vascular aspect and is therefore fit for various tests done so far on animals. Experimental data has proved that 2 cm² of LSE, if exposed to different chemical factors causing skin or cornea irritation, reacts in the same biological way as natural skin and releases pro-inflammatory mediators: prostaglandins E₂, prostacyclin, interleukins^[6, 7]. It is also of great importance that while using LSE, we can test solid substances, insoluble substances, liquids, emulsions and creams applied locally to places exposed to air. We can also study the adhesion mechanisms of microorganisms which can

infect the skin and the effect of drugs on these microorganisms. We can also test new drugs on LSE, drugs which, e.g. contain pharmacologically active bee products.

Currently it is also possible to reconstruct in laboratory conditions other cellular models, which show characteristics of an organ particular cells come from. This is how veins and small liver lobule are created. The organotypic culturing is especially interesting in the aspect of studying the mechanisms of positive influence on a human organism of products deriving from flower nectar and honey dew.

In many researchers and practicing doctors' opinion, it is immunotherapy – which purpose is to strengthen the natural protection system of an organism using immunomodulators – that is becoming more and more important. The actual positive influence of bee products on the immune system can no longer be doubted. It has been known for a long time that propolis increases an organism's immunity and that apitoxin strongly stimulates the immune system. All that is left to us now is the process of experimental trials and errors. Using phytohemagglutinin as a mitogenic factor makes it possible to culture lymphocytes. Two different methods are used for this purpose – macroculturing and micromethod. The macroculturing requires collection 5-10 ml of blood and consists in culturing lymphocytes, isolated either by centrifugation or sedimentation. The micromethod consists of culturing a small amount of cells in 0.3 - 0.5ml of complete blood. If we analyze closely the attitudes realized in the biomedical sciences, the need to get to know the human transcription is apparent. That is why it is purposeful to know the sequences written over from mRNA to sDNA and to construct expressive cDNA libraries – it will enable efficient cloning of only the region of the gene, and not all the other genomic sequences around it.

The classical, but also the more modern methods of molecular biology make it possible to see the differences in genes expression on the transcriptive level. Genes of diverse expression had been until recently identified only by means of differential or subtractive hybridization and separation of their protein products. Since the time we started to be able to use resourceful databases of nucleic acids and proteins sequences, the number of genes which expression we can follow has outgrown the capacity of traditional analyses.

One of the modern approaches to diverse analysis of gene expression based on the hybridization techniques are the DNA microprocessors. The technology behind it comes down to immobilizing on a ground a fixed carrier of a large number of single-strand oligonucleic or DNA particles^[8, 9]. These particles play the role of molecular probes, although they do not contain markers. It is the deoxy- and ribonucleic acids which hybridize with them that are analyzed and marked. Thanks to DNA microarrangements it is also possible to study the binding of DNA and proteins or other ligands. Sensitive techniques of fluorescence detection make it possible to identify precisely each component of the studied mixture and to evaluate quantitatively its binding. Obtained data is collected and worked out with advanced computer software. This strategy enables the analysis of thousands of genes and evaluation of their expression in an impressively short time in the process of a single experiment. The DNA microarrays are already more and more popular in studies on individual sensitivity to a drug, optimization of their effectiveness and in looking for new drugs; they are still, however, very costly.

Additional opportunities awaiting DNA microprocessors can be easily seen in the studies of interactions between different substances and their natural composition with the genetic material as well as regulation of genes' activity within a cell. Since it has been proved that in chemotherapy some of the long-used bee products show synergistic effects, adding them to this treatment enables us to lower the doses of some drugs and to widen the spectrum of their activity.

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