JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, 17(1), 98-104

Journal of Agroalimentary Processes and Technologies

Animal models used in the investigation of xenobiotics of food interest and pharmaceutical interest

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Received: 20 February 2011; Accepted: 20 March 2011

Abstract

Using of animal models in the study of the effects induced by chemical xenobiotics is of great importance in life sciences and in the bioscience-food science relationship. Experimental investigations on animal models (predilectly mammals), i.e. in vivo, allowed to elucidate the specific molecular mechanisms of "nutrients" metabolization, respectively of "xenobiotics" biotransformation, and the possible interactions among their intermediate products ("metabolites", respectively "xenobioderivatives") some of them with implications in pathogenesis. On the whole, such studies are of interest for metabollomics.

Among the studied xenobiotics there are also included the organometallic compounds concerning metallomics. Investigations on the organo-metallic compounds are of special interest in researches having application in the study of cytostatic chemotherapeutics.

Studies on animal models of the known xenobiotics in the bio-anorganic chemistry had in view the effects induced by non-nutritive compounds, synthetic chemotherapeutics, some cosmetic ingredients etc. In this framework there are discussed the characteristic effects triggered by xenobiotics of food interest and pharmaceutical interest. In the peculiar case of organo-metallic compounds one can have a view on their effects on the biochemical homeostasis, on the status of the hematopoietic system and even on the morphology of various tissues.

Keywords: chemical xenobiotics, studies on animal models

1. Introduction

The use of animal models in biochemical investigations applied in nutrition, pharmacology, ecology etc. has an important role in life sciences [25,30,52].

In the case of studies related to xenobiotics the animal models are involved in assessing their effects on living organism having in view the mechanism of action, routes of excretion etc. [32,50,33]

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Chemical xenobiotics are in fact chemical substances foreign to life and include environmental contaminants, food contaminants, synthetic drugs, some ingredients of cosmetics a.o.

In the organism the physiological processes may evolve in two directions: metabolization of food nutrients and biotransformation of xenobiotics (from food, drugs, cosmetics, environmental pollution). Nutrients resulted in the organism by food intake undergo "natural biochemical pathways" (metabolization through catabolic and anabolic processes) while xenobiotics undergo "specific biochemical pathways" (xenobiotransformation processes through xenobiodegradation and xenobiosynthesis) [14].

With respect to nutrients, in the first phase they are degraded into smaller units or metabolites through biodegrading processes known as the catabolism and in the second phase the metabolites are used at the cellular and tissular level to synthesize essential molecules through processes of biosynthesis known as anabolism.

With respect to xenobiotics, they undergo biotransformation processes in two distinct phases: xenobiodegradation and xenobiosynthesis. The xenobiodegradation phase involves oxidoreduction and hydrolyses reactions while the xenobiosynthesis phase involves conjugation and adductation reactions [13,34].

Xenobiotics of food interest and pharmacological interest one may underline the fact the chemicals used for food processing in the food industry, the new drugs produced by the pharmacological industry and in generally al the xenobiotics to which humans may be exposed must be tested on animals in order to assess their safety.

However the animal models cannot predict all of the reactions a human may have to a given substance one have the possibility to obtain general information about the physiological (physiopathological) effects of that substance on various organs (heart, kidney, liver, brain, lungs etc) and to determine the dangerous (harmful) doses. The effects caused by a given substance on organs and tissues are triggered by pathobiochemical lesions at molecular level.

1. Animal models - general principles

The animal models may be used during the research and investigation of various human disease, in the safety assessing of various xenobiotics (food additives, food contaminants, environmental pollutants, drugs etc), in order to determine the efficiency of new drugs or treatments etc. The animals that are chosen must usually meet a determined taxonomic equivalency to humans (to resemble human physiology as much as possible). A classic definition given by Wessler (1976) states that an animal model is "a living organism with an inherited, naturally acquired or induced pathological process that in one way or another closely resembles the same phenomenon in man".

The animals of choice in scientifically research may be both invertebrate and vertebrate animals. The invertebrate models are usually used in fields like genetics, neurobiology etc., while vertebrate models are preferred and are used mostly in the fields where animal models can be used [5]. The vast majority of animals used in research are in the fields of pharmaceutical research (new drugs development and testing) and in food science and technology (testing of non-nutritional food ingredients, food packaging materials a.o.).

From the vertebrates models, rodents are the animals most used in modern research, not only due to their low cost and maintenance, or to the similarities between their physiology and the physiology of humans but also because they have a naturally short life span (two to three years) which allows scientists to easily observe what happens during the progress or pathogenesis (pathobiochemistry, pathophysiology) of a disease. Advances in genetic engineering have made possible the development of some excellent rodent models for research. The availability of "transgenic mice" (which have added genes) and "knock-out mice" (which have disabled genes) have had a big impact in the research and understanding of cancer, Parkinson's disease, cystic fibrosis, heart disease, memory loss, muscular dystrophy, spinal cord injuries etc. [5] The so-called "nude mouse" – lacking a functioning immune system has become a very important model in understanding cancer suppression. Also there are various transgenic animals which have new genes inserted, or specific genes modified or removed in order to mimic specific disorders like Huntington disease [38], diabetes [39], Alzheimer [19] etc.

Animal models that use rats are preferred in some fields due to their natural resistance to diseases and also to infections, an important aspect in the experiments that requires surgical interventions.

2.Investigations on some xenobiotics of food interest

When discussing the xenobiotics of food interest, one must take into account especially food additives (their use are considered as deliberate contamination of food) and food pollutants (pesticides, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins etc). In either cases safety or toxicological tests are done using animal models.

In the case of food additives, their safety is determined through extensive testing on animal models before the authorities approve them. For an additive to be approved, animal toxicity and metabolism/biotransformation studies of the additive must supply substantial information covering the following areas: identification of hazards posed by the additive; indication of the dose-toxicity relationship for those hazards, estimation of the probable human consumption of such additives. The determination of the NOEL (no observed effect level) or the NOAEL (no observed adverse effect level) from animal toxicity studies is very important [8]. These parameters are determined through chronic toxicity or lifetime exposure studies to the additive. The NOEL or NOAEL, measured in terms of the weight of the additive per kg body weight per day, will be used to determine the ADI (acceptable daily intake) for humans. The ADI is a parameter that reflect the amount ingested over an entire lifetime and it is usually set at 1% of the NOEL or NOAEL because it takes into account the possibility that the additive has greater toxicity in humans compared to experimental animals and also it takes into consideration the increased susceptibility of specific members of the human population [51].

The safety of some xenobiotics of food interest (especially food additives) is usually investigated by changing the administered dose of a single xenobiotic in animal models or cell cultures [44].

Examples of additives tested on animals are abounding in the scientific literature.

Such studies were made in case of food sweeteners like aspartame tested on rats [35] and Swiss mice [43] and other artificial sweeteners (saccharin, cyclamate based, acesulfame-K based, and aspartame) tested on mice [36]. Other studies made on mice investigate the effects of sodium glutamate [24] or the reproductive and neurobehavioral effects of tartrazine [46,47]. The toxic effects are severe usually only in the case of artificial additives, but there are some exception. One exception is discussed in a 90 day toxicity study made on rats with a natural additive (montan wax) which shown the potential of inducing multiple granulomas in liver [21].

In biochemistry and pathobiochemistry a peculiar importance has the biogenesis of adducts formed between DNA and small molecules or between DNA and metallic ions that accede with various nutrients, that are in fact the biometals (e.g. Mg, Ca, Zn, Mn etc). In the case of the food xenobiotics the adducts biogenesis often regards toxic compounds as polycyclic aromatic hydrocarbons (PAHs), nitrosamines, mycotoxins, metallic ions with toxic potential (e.g. Al, Sn, Pb, Hg, Cd etc) [16].

Food pollutants represent an other class of xenobiotics of food interest which are often studied and tested on animal models. Some of the most problematic food pollutants are pesticides. Because human responses to a pesticide cannot be mimicked exactly or modeled by a single animal species, it is often necessary to use multiple species - rats, mice, rabbits, guinea pigs, dogs - to determine the pesticide toxicity to humans. To animals is usually administered a pesticide dose via the predominant route of expected human exposure: oral; dermal; inhalation [3]. The researches conducted on animals often showed that pesticides possess neurotoxic effects [32] and presumably may be involved in neurodegenerative processes like Parkinson disease [6]. An other important class of food pollutants is represented the polycyclic aromatic hydrocarbons (PAHs). Studies made on mice had shown that the administration of PAHs prior to pregnancy and/or during lactation compromised the ovarian reserve of female offspring [22] and also that PAHs administration trigger fetal growth restriction which is associated with altered placental vasculature and AhR-dependent changes in cell death [7].

Metals and organometallic compounds are often found in foods as pollutants that accede from the environment.

Researches regarding the mechanism of action for various organometallic compounds on biological systems were often undertaken using animal models and studying the modifications of the biochemical homeostasis [9, 10,42]. Investigations on the action of some metals found in excess (Zn, Mn) and of a metal with toxicological potential (Al) were the subject of some extended researches made on animal models [48,37].

Some classes of xenobiotics can be found in food both as environmental contaminants and as additives. Nitrates and nitrites are an eloquent example – they are found as contaminant in fruits or vegetables due to the fact that nitrates are used as fertilizers and in meat due to the fact that animals can drink contaminated water or consume contaminated fodder. Also nitrates can also be added in meat products as additives with the role of keeping the reddish color of the meat and to better preserve the products. Experiments on the effect of nitrate consumption were made on various animal models. Such an experiment was made on rabbits (family Leporidae) and investigated the effects on the biochemical homeostasis after the administration of nitrates in drinking water [17].

Other xenobiotics of food interest are the genetically modified foods because often by changing the genome of animals and plants, the modified organism introduce in our diet new substances which may be considered xenobiotics. Therefore it is important to test their safety and animal models are vital to this kind of testing [41,54,55].

One of the effects that are usually related to genetically modified food consumption is the increase of allergies, therefore the animal test often are focused on the allergenicity of the new genetically modified organism. There were made studies in order to determine the allergenicity of genetically modified soybean protein extract on mice [18], the effects of transgenic maize on the mice immune system.

3.Investigations on some xenobiotics of pharmacological interest

In the case of animal models that are used for drug testing, is important to use the minimum number of animals necessary to arrive to scientifically reliable data and also to ensure the human and proper care of animals.

Generally, two or more species are tested because a drug may affect one species differently from another.

One of the main uses of animal models in pharmacology is the assessment of the treatment efficiency and of the various toxic effects that may occur. Some examples of experiments using animal models for this purpose are given below. The citostatic activity of 2-methoxyestradiol was studied in an orthotropic rat glioma model [23].

Flavopiridol, a semi synthetic flavone derivative of the alkaloid rohitukine known to inhibit potently the activity of multiple cyclin-dependent kinases, was assessed in mouse models of localized and disseminated human hematopoietic neoplasms and has shown potent antitumor activity [2].

The preventive effect of finasteride on chronic bacterial prostatitis (CBP) was assessed using Wistar rats and the results suggested the possibility that finasteride has a preventive effect on the development of this disease [26]. The cytotoxicity and antitumoral activity of dichloromethane (a compound extracted from the aerial parts of Pothomorphe umbellata) was evidenced using Swiss mice [40].

Besides treatment efficiency, animal models may also be used to obtain data about how much of a drug is absorbed into the blood, how is it metabolized, the toxicity of the drug and its breakdown products (metabolites), and how quickly the drug and its metabolites are excreted from the body. In other words, animal models are also used to acquire pharmacodynamic and pharmacokinetic data.

In order to offer an overview on the importance of animal models in this field there will be exemplified some xenobiotics of pharmacological interest studied on animal models as follows. Metabolism and excretion of rivaroxaban, an oral, direct factor Xa inhibitor for the prevention and treatment of thromboembolic disorders, was studied and determined in rats, dogs, and humans [49].

The uptake, transport and regulation of cyclo-trans-4-l-hydroxyprolyl-l-serine (a dipeptide with anti-hepatitis activity) were assessed in rat models [27]. Pharmacokinetics, metabolism, and excretion of anacetrapib (an inhibitor of the cholesteryl ester transfer protein) were assessed in rats and rhesus monkeys; the novel drug revealed a low clearance in both species and a moderate oral bioavailability [45].

Studies regarding the action of xenobiotics of pharmaceutical interest include synthesized chemotherapeutics belonging to a certain class of chemical compounds.

Also, one can study chemotherapeutics belonging to different classes of chemical compounds but are used for the same group of disease, e.g. neuromuscular diseases, cerebrovascular diseases, various forms of cancer.

The problem of DNA adducts is important not only in pathobiochemistry regarding the mutagene and oncogene processes but also in pharmacotherapy regarding the mechanisms through which the cytostatic works [15]

Among important compounds from pharmaceutical point of view are the organometallic ones. Some of these chemical xenobiotics, especially the complexes of Pt (II), and Pt(IV), are used in the cytostatic chemotherapy. Nowadays in the clinical practice only cisplatin (cDDP) is administered. The effects of this cytostatic drug were studied first on micro-organisms and subsequently on laboratory animals, i.e. mammals [11,12] and in clinical trials [28]. More extended researches on cisplatin, targeting predilectly the dyshomeostasis of protein metabolism (DNA, non-protein nitrate metabolites) and hydroelectrolyic metabolism undertaken at the level of various tissues, e.g.: hepatic, renal etc. [31]. But, experimental researches are performed also on cytostatic organometallic compounds containing Fe, Co, Ga, Au, Sn etc. [1,4,20].

Also, the use of animal models in the field of pharmacology may allow to elucidate the possible interactions between various drugs, interactions which may lead to antagonistic or synergistic effects. Such an example is the recent study on the effects of myricetin (an anticancer compound) on the bioavailability and pharmacokinetics of tamoxifen and its main metabolite, 4-hydroxytamoxifen in rats, a study that revealed the fact that myricetin increases the bioavailability of tamoxifen but it decreases the formation of 4-hydroxytamoxifen [29].

The effects of a treatment using a combination of implantable poly (D,L-lactide-co-glycolide) microparticles of Temozolomide and vatalanib were studied on a rat orthotopic glioma model and the results revealed that combination treatment with both of them had exhibit an inhibitory effect to rat glioma tumors, a significant decrease in cell proliferation, an increase in apoptosis, and a lower microvessel density within the glioma tumors while improving the survival time versus single agent therapy [53].

Concluding remarks

In vivo studies, i.e. on animal models,upon the chemical xenobiotics action permit the elucidation of the molecular mechanisms of the biotransformation of the chemical xenobiotics of food interest and pharmaceutical interest. An apart aspect is represented by metabolomics which can clarify the occurred interactions between the "metabolites" resulted from the nutrients metabolization and the "xenobioderivatives" resulted from the biotransformation of xenobiotics.

Interactions occurred between xenobioderivatives and metabolites can permit the evaluation of the changes in the chemical homeostasis of proteins, lipids, carbohydrates and biominerals.

The evaluation is carried out by bioanalytical investigations – specific to clinical laboratory in order to have a more exact image on the status of some usual "biochemical markers". Also, one can follow up the bioaccumalative effects with repercussions on blood and organs. In some cases the investigations leaded to the finding of some "xenobiochemical markers". Such situations can find in case of metals with toxic potential and in case of biometals in excess (as compared with the physiological needs). Thus the information on the effects induced by the chemical xenobiotics may permit the establishment of the limitative relationship between biochemistry-pathobiochemistry-pharmacotherapy.

Acknowledgements.
This work were elaborated with the financial support of the Romanian Ministry for Education, Research, Youth and Sports – National Authority for Scientific Research, PN – II - ID – PCCE – 2008 – 1 (Project Nr.2 / 2010, code CNCSIS ID–PCCE 140) entitled "Biomedical application of metal compounds - Metallomics").

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