METABOLIC ACTIVITY OF GUT MICROBIOTA AND XENOBIOTICS

ABSTRACT: The intestine habitat is the natural collection of symbiotic microorganisms. The bacterial population enables many permanent metabolic activities in this environment. Inside the intestine of mammals there are an extended genome of millions of bacterial genes named microbiome. In recent years, there has been an increased interest of scientists to discover the place and the role of bio-ecological content and modulation of gut microbiota in a host organism using prebiotics, probiotics and synbiotics, which may have a great benefit for human health.

KEYWORDS: gut flora, metabolism, xenobiotics

INTRODUCTION

Metabolism is a general term used for chemical transformation of xenobiotics and endogenous nutrients (e.g., proteins, carbohydrates and fats) inside or outside the host. Xenobiotics are all classified as chemical substances that are foreign to the host. They are not nutrient to the body and they enter it through ingestion, inhalation or dermal exposure (drugs, industrial chemicals, pesticides, pollutants, plant and animal toxins, etc.) [Mariat et al., 2009; Furet et al., 2010; Ley 2010]. The liver is built up of the endoplasmic reticulum (predominantly smooth endoplasmic reticulum) and other tissues which contain a

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large complex of enzymes, together called microsomal enzymes (microsomes are small spherical vesicles derived from endoplasmic reticulum after disruption of cells by centrifugation, and the microsomal enzymes are present in microsomes) [Kleessen et al., 2005; Sartor, 2004; Jia et al., 2008; Palmer et al., 2007]. Microsomal enzymes catalyse glucuronide conjugation, a great part of oxidative reactions, and some reductive and hydrolytic reactions. The factors which affect drug metabolism are: a) species differences (procaine, barbiturates, etc.), b) genetic differences (there are variations among species), c) age (faetus, newborn, old), d) sex (as a reflection of sex hormones), e) nutrition (some kind of starvation and malnutrition), and f) pathological conditions (of liver or kidney). The human intestinal microflora is composed of different microbiota. There are near $10^{12}$ organisms per gram of gut content. This environmental circle supports activities of the gut flora as a huge metabolic system. The influence of resident gut microbes on xenobiotic metabolism has been investigated at different levels throughout the past five decades [Bojić Milićević et al., 1996; Bojić Milićević et al., 1995; Bojić Milićević et al., 2005; Bojić Milićević et al., 1998; Bojić Milićević et al., 1996; Bojić Milićević et al., 1997]. The investigations confirmed the influence of microbes on xenobiotics, which can have direct metabolic effects on toxins and other xenobiotics by conventional culture-based techniques, and explained the role of community composition on drugs metabolic profiles through DNA sequence-based phylogeny and metagenomics. This modern view of analysis opens new horizons for research how microbiome compositional and functional variations affect drug action, fate, and toxicity (pharmacomicrobiomics) in human intestine. The key role of these researches has been to describe the microbial communities associated with the human gut, to determine whether there is a common gut microbiome profile shared among healthy humans, and investigate the effect of its changes on health [Hamer 2008]. The explanation of pathways how the microbiome interacts with human metabolic enzymes in the liver and intestine is of the highest interest. The evolution of the complex metabolic interaction between intestinal microbiota in the human gut with its host is multidimensional. The composition of the intestinal microbiome is initially determined by genetic and environmental moments. In fact, external influences like host immune response provide the equilibrium in health and disease. The metabolism of drugs by intestinal bacteria and further by enterocytes with absorption into portal system may give a better understanding of pre-systemic drug metabolism, delivery, and toxicity [Salminen et al., 1998].

The gut microbiota is the most predominant and most diverse microbial community in human body. There are hundreds of microbial species, about 10 times more than the number of body cells. Human metabolic processes can reach up to 36% of small molecules in human blood as a treasure of the gut microbiome [Wilson et al., 2009; Ley et al., 2007; Costello et al., 2009]. The gut microbiota is involved in drug metabolism and has been explored since the middle of last century. One of the best definitions of the term microbiome was first suggested in 2000 by Joshua Lederberg, a Nobel Prize Laureate, to explain the participation of a large number of microbial genomes associated
with the human body. He made an approach to microbiome as a part of the “human extended genome”. The term *microbiomics* refers to the functional aspects related to the microbiome. Pharmacogenomics explains the effect of human genome variations on drug disposition and reaction. Gut microbiota has a significant role and very much influence the metabolism of xenobiotics according to response-modifying process, as in several explored mechanisms. Drug metabolism can be changed by gut flora activity directly by producing enzymes that degrade or activate the drug molecules, or in a special game of the competition with drug molecules crossing by the metabolizing enzymes [Manichanh *et al.*, 2008; Ley *et al.*, 2006]. The gut microbiota also may affect drugs by modulating the activity or exchanging the levels of the host’s drug metabolizing enzymes or by a complex process of producing enzyme. As the consequence of it there are brand new metabolites, originally derived from diet in germ free mice. This circle and activity of huge gut microbiota is in correlation with changes in quantity of liver and intestinal metabolic enzymes before their corresponding levels in mice with conventional gut micro flora ecosystem. The conventional gut microflora in human and mice are proved to be associated with a modest game in the levels of drug-metabolizing enzymes, for example sulfotransferase 1 B1 (SULT1B1). Nicholson et al. tried to make postulates of interaction of host and microecosystem in xenobiotic metabolism in the hindgut by making an interesting model, assuming six different kinds of cells in host and microbiome, each kind of which has its own transcriptome and metabolome depending on its place and change thorough special pathways. It was confirmed a common metabolic pathway between host and microbiota [Sartor 2004; Zoetendal *et al.*, 1988]. The external component is human metabolome (the sums and interactions of all the cellular metabolomes). The production of indole-3-propionic acid was shown to be completely dependent in the presence of gut microflora, and could be organized by colonization with the bacterium *Clostridium sporogenes*. A lot of organic acids with phenyl groups were also greatly increased in the presence of gut microbes. Different factors as diet, genotype, and microbial interactions contribute to the diversity, but also to the relative balance of the intestinal microbiome. The two bacterial phyla Firmicutes and Bacteroidetes are dominant among the bacterial population of the intestine. The discovery of this diversity has been made possible by using advanced analytical techniques such as 16S rRNA-targeted oligonucleotide fluorescent probes or mass spectrometry and nuclear magnetic resonance methods. The implementation of these methods provides the differentiation among the microbial flora of each individual, reducing the spread of other species and strains [Ley *et al.*, 2005; Turnbaugh *et al.*, 2009; Paster *et al.*, 2001].

**MICROBIOME – HOST INTERACTIONS**

The understanding that the intestinal microbiome interacts with the host has been recognized in different areas. There are considered symbiotic interaction for optimal food processing and local environment for optimal growth.
conditions of dominant species, as well as immune interactions that benefit the host and keep a healthy mucosal barrier between intestinal bacteria and the host. The symbiotic interaction between host and microbiome for food processing and digestion is involved in bile acid metabolism and enterohepatic recycling of drugs and some nutrients like supplements of food. Those symbiotic interactions start very soon after birth and remain in the healthy population although an acute disease or disorder of bacterial equilibrium as a consequence of antibiotic therapy may produce chronic disorders that can constantly affect the ability of the host to process food optimally. Some bacterial strains play a very important role in the metabolism of nutrients such as choline or taurine and are essential for the absorption of fatty acids by the host [Pei et al., 2004]. While gut bacteria mainly use sugars for energy production leading to the formation of short-chain fatty acids such as acetate, propionate, and butyrate, the host utilizes these metabolic products for its energy consumption – muscles, heart, and brain utilize acetate while butyrate is important for enterocytes. Bacteria in the gut provide the host with essential amino acids and form a large number of vitamins like A, K and biotin. They also take part in biotransformation of bile. It is confirmed that the host genotype contributes to the diversity and specific composition of some bacterial species. Environmental factors such as diet and initial colonization after birth have a stronger influence.

**ENTEROCYTE METABOLISM**

It is proved that enterocytes are expressed in CYP 450 enzymes, especially CYP 3A4 and CYP 2C family members as well as phase II metabolizing enzymes like UDP-glucosyltransferase (UGT) and sulfotransferase. In fact, phase II conjugation enzymes and sulfotransferase activities are nearly 250–300% higher in the jejunum compared to the liver. The absorption as well gut wall metabolism of drugs depend on the mechanisms belonging to the hepatic metabolism. A lot of drugs first have to come inside the enterocytes in the beginning of metabolism game, mainly CYP 3A4 substrates. Permeability of drug compound is additional factor which plays an important role in drug metabolism [Bik et al., 2006; Eckburg et al., 2005]. Lipophilic compounds are readily absorbed in the gut and they are ready to be involved into metabolism. In that way, hydrophilic substances may require special active transport systems that limit their absorption. The influence of microbiota on metabolic processes has been known for decades, but the interest in pharmacokinetic and toxicokinetic influence on drug metabolism has grown recently. There are a lot of examples where absorption and metabolism are influenced by sometimes even crucial components of biodegradation of drugs [Eckburg et al., 2005]. The bioactivation is the reductive metabolism of sulphasalazine usually used in the treatment of ulcerative colitis which is classified as chronic disease. Also, prontosil and neoprontosil, pro-drugs of sulfanilamide, are metabolized in the large intestines to release the active sulfanilamide drug. This kind of metabolism was later proved for aminosalicylic acid pro-drugs like olsalazine.
which results in anti-inflammatory effects in the gut as well as absorption and excretion of the corresponding coupling agent and the salicylic acid derivative [Hooper et al., 2001; Tlaskova-Hodgenova et al., 2004; Round et al., 2009]. Bioactivation is the example of plenty beneficial effects of microbial metabolism. In some cases metabolic activity of gut microbiota may result in forming of toxic metabolites with local and systemic effects [Haverson et al., 2007; Stappenbeck et al., 2002; Lefèbvre et al., 2009; Wong et al., 2006]. The reduction of nitrazepam by gut flora is proved both in rat and human intestinal tract by 7-aminonitrazepam. It is then further metabolized in the liver after absorption of 7-acetylaminnitrazepam, which is known as teratogenic metabolite. Notable toxicity due to bacterial metabolism in the intestine is related to the bone marrow aplasia of a metabolite of the antibiotic chloramphenicol, which can happen in 1% of patients after oral consumption of chloramphenicol and also refers to gut microbiota. In such cases in small intestine there are a high percentage of coliform bacteria that are able to transform chloramphenicol to a toxic metabolite p-aminophenyl-2-amin-1,3-propanediol. Particularly common metabolic reaction that has an important role in fecal drug excretion and enterohepatic recirculation is a deconjugation reaction occurring in the intestinal tract as in the case of paracetamol [Hapfelmeier et al., 2010; Atarashi et al., 2011; Gibson et al. 2010; Cani et al., 2009]. A great number of conjugated drugs like glucocorticoids, morphine, indomethacin and sex hormones are excreted via bile as glucuronic or sulfate acid metabolites. Bacterial metabolism of these conjugates consequently form aglycones or desulfated compounds that can be reabsorbed in order to prolong their biological half-life [Larsen et al., 2010; Ley et al., 2010]. There is a number of drugs that are mostly metabolized through phase II sulfation pathways like tamoxifen and apomorphine, among others [Roberfroid et al., 1995; Elmer et al., 1996; Sadler et al., 1998; Neut et al., 1980; Woese 1987]. This activities of gut flora metabolism show an important impact of drug metabolism and toxicity. Metabonomics and gene sequencing can overcome the obstacles and allow for a more comprehensive picture of both intestinal drug metabolism and mechanisms of toxicity [Stahl et al., 1988; Rama et al., 2012; Nicholson et al., 2003].

**CONCLUSION**

Physiological state of a host depends on the intestinal microbiota. Gut microflora has a huge influence on many pathways of metabolic activities and pathways of drugs and xenobiotics. The habitat of intestine provides survival of beneficial bacteria, which contribute to biotransformation of consumed xenobiotics to therapeutic and toxic products that could be released and reabsorbed by enterohepatic recirculation. The future can bring new knowledge about further gut flora role in almost any metabolic activity of the compounds and modern methods of the determination of bacterial genome as the key point in interaction between gut flora and host.
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REFERENCES


САЖЕТАК: Цревни тракт је природно станиште симбиотичних микроорганизма. Бактеријска популација омогућава перманентно одвијање многих метаболичких активности у овом еколошком окружењу. Сисари су наоружани обимним геномом милиона бактеријских гена унутар црева познатих под називом микробиом. Последњих година порасао је интерес научника за откривање места и улоге биоеколошког садржаја и модулације цревне флоре у организму домаћина применом пребиотика, пребиотика и синбиотика, који могу корисно да утичу на људско здравље.

КЉУЧНЕ РЕЧИ: цревна флора, метаболизам, ксенобиотици