Human gut microbiota plays a role in the metabolism of drugs

Lenka Jourova^a, Pavel Anzenbacher^b, Eva Anzenbacherova^a

Background and Aims. The gut microbiome, an aggregate genome of trillions of microorganisms residing in the human gastrointestinal tract, is now known to play a critical role in human health and predisposition to disease. It is also involved in the biotransformation of xenobiotics and several recent studies have shown that the gut microbiota can affect the pharmacokinetics of orally taken drugs with implications for their oral bioavailability.

Methods. Review of Pubmed, Web of Science and Science Direct databases for the years 1957-2016.

Results and Conclusions. Recent studies make it clear that the human gut microbiota can play a major role in the metabolism of xenobiotics and, the stability and oral bioavailability of drugs. Over the past 50 years, more than 30 drugs have been identified as a substrate for intestinal bacteria. Questions concerning the impact of the gut microbiota on drug metabolism, remain unanswered or only partially answered, namely (i) what are the molecular mechanisms and which bacterial species are involved? (ii) What is the impact of host genotype and environmental factors on the composition and function of the gut microbiota, (iii) To what extent is the composition of the intestinal microbiome stable, transmissible, and resilient to perturbation? (iv) Has past exposure to a given drug any impact on future microbial response, and, if so, for how long? Answering such questions should be an integral part of pharmaceutical research and personalised health care.

Key words: microbiome, metabolism of drugs, gut microbiota, bioavailability, cytochromes P450

Received: April 26, 2016; Accepted: July 13, 2016; Available online: August 2, 2016
<http://dx.doi.org/10.5507/bp.2016.039>

^aDepartment of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry and Faculty Hospital Olomouc, Palacky University Olomouc, Czech Republic

^bDepartment of Pharmacology, Faculty of Medicine and Dentistry and Faculty Hospital Olomouc, Palacky University Olomouc, Czech Republic

Corresponding author: Eva Anzenbacherova, e-mail: eva.anzenbacherova@upol.cz

INTRODUCTION

The human body carries trillions of microorganisms, whose aggregate genomes have substantial metabolic potential and are referred to as the microbiome¹. The largest number of these microorganisms resides in the gut and is termed the gut microbiota. Recent studies have shown that the commensal intestinal flora have profound effects on overall health of the host for which it performs various essential biological functions such as synthesis of vitamins², development and modulation of the immune system, bacterial defense³, the intestinal response to epithelial cell injury⁴ and nutrient metabolism⁵. Changes in the structure and diversity of the microbiome are also associated with large array of pathological states such as inflammatory bowel diseases (IBDs) (ref.⁶), metabolic diseases such as obesity and diabetes⁷, atherosclerosis⁸ and cardiovascular disease⁹. The gut microbiome is relatively stable but its composition and/or function may be influenced by a range of factors such as diet¹⁰, probiotics¹¹, and drugs, especially antibiotics¹². Gut microbiota also affect the metabolism and toxicity of xenobiotics. They express a diverse array of bacterial enzymes which have the ability to metabolize drugs far more extensively than any other part of the body^{13,14}. Orally taken drugs reach the gastrointestinal tract and before being absorbed through the epithelial membrane into the blood they are exposed to

the intestinal microbiota that may alter their disposition, efficacy and toxicity. Thus, the effect of intestinal microbiota can have significant implication for the oral bioavailability and half-life of drugs¹⁵. However, understanding of the effect of the intestinal bacteria on the stability of the drugs in the gastrointestinal environment remains limited.

COMPOSITION OF THE GUT MICROBIOTA

Our understanding of the composition and functions of the human gut microbiota is crucial to assess their metabolic potential and influence on human health. Over the last two decades, knowledge of this area has increased enormously, mainly due to the next-generation sequencing technologies and metabolite profiling. The majority of microbes in our bodies reside in the gastrointestinal tract and provides a wide range of interactions with the host¹⁶. In addition to bacteria, the gut microbiota consists of archaea, yeasts and filamentous fungi¹⁷. Recent studies have suggested that the viral component, including a variety of bacteriophages, is also important¹⁸. Gut microbiota are an extremely diverse bacterial community (approximately 500 species per individual), however, the gastrointestinal tract of a healthy adult is typically represented by two dominant bacterial phyla, the gram-positive *Firmicutes* and gram-negative *Bacteroidetes* along with lower abundances

of *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia*¹⁹. *Bacteroides* is the most abundant and the most variable genus²⁰. The human gastrointestinal tract is sterile in the prenatal period. During and immediately after birth, initial microbial exposure occurs. While, vaginally delivered infants are colonized by maternal vaginal and faecal microbiota, dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp, infants born via caesarean delivery are instead, colonized with microbes associated with the skin, dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. and with the hospital environment^{21,22}. The genus *Bifidobacterium*, belonging to the phylum *Actinobacteria*, is a dominant microbial group in healthy breastfed babies. The role of this genus in gut homeostasis is summarized in a published review²³. Despite great individual variability, the gut microbiome of adults is considered to be relatively stable. According to Arumugan et al., 2011 the human population can be divided into only three enterotypes, identifiable by variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3) (ref.²⁴). Also during senescence, the composition of the gut microbiota undergoes new changes. Claesson et al. has found that elderly people differ from the core microbiota and diversity levels of younger adults, with a greater proportion of *Bacteroides* spp. and distinct abundance patterns of *Clostridium* groups²⁵.

Nevertheless, describing "the normal" gastrointestinal microbiota residing within healthy humans is still very challenging since most are anaerobic and uncultivable and also because of enormous individual differences.

FACTORS INFLUENCING THE COMPOSITION AND FUNCTION OF THE MICROBIOME

The human gut microbiota is one of the most complex ecosystems on the planet and many studies are focused on the investigation of the composition and function of the gut microbiota and their interaction with the host^{26,27}. Imbalance in the composition of this bacterial community can lead to transient intestinal dysfunctions and pathological states²⁸. In recent years, an increasing number of studies on the impact of several classes of drugs, xenobiotics and also dietary plant substances on the composition of the gut microbiota have been published²⁹. The microbial intestinal metabolism is adversely affected especially by antibiotics³⁰. According to recent studies, antibiotics produce drastic short- and long-term alteration and disruption of the gut microbiota which may lead to the partial decrease in the overall diversity of the microbiome³¹. On the other hand, different studies indicated the plasticity of the gut microbial composition which can be reshaped after discontinuation of antibiotics^{30,32,33}.

However, the relationship between the composition of the gut microbiome and host organism is mutual and many recent studies have focused on this unique interaction. To date, a myriad of papers have reported the association of the activity of gut microbiome with obesity and diabetes mellitus^{7,34,35} asthma and allergy³⁶⁻³⁸, aging^{39,40} and

even the development and function of the central nervous system⁴¹, autism and depression⁴².

METABOLISM OF XENOBIOTICS

With 100 trillion microbes encoding 100-fold more unique genes than our own genome and secreting a diverse array of bacterial enzymes, the gastrointestinal tract has enormous genetic and metabolic potential⁴³. The ability of the gut microbiome to metabolize drugs was first recognized over 40 years back^{44,45}. To date, dozens of drugs and diet-derived bioactive compounds have been reported to undergo direct microbial modification⁴⁶⁻⁴⁹, and their number is still growing. In almost all cases, the exact mechanism, the specific reaction and the responsible microbial species remain unknown. However, several mechanisms have been proposed and demonstrated⁵⁰. Currently, new sequencing and pyrotagging technologies allow new insights for answering the questions how gut microbiome affect the disposition, efficacy and toxicity of drugs⁵¹. Several recent studies have shown that the gut microbiota can influence the pharmacokinetics of orally administered drugs and, thus, may have significant implications for their oral bioavailability^{52,53}.

DRUGS METABOLIZED BY THE GUT MICROBIOTA

Azo reduction

The first reported example of the azoreduction of drugs by gut microbiota was modification of orally administered **prontosil** and **neoprontosil** to sulfanilamide. Concurrently, this conversion was the first known prodrug activation by gut bacteria, as metabolite sulfanilamide was found to have antibacterial effect. Azoreductases produced by the large intestinal microbiota are responsible for the reduction of the azo bond⁵⁴. Prontosil is metabolized to sulfanilamide also in the liver and kidney (Fig. 1.) (ref.⁵⁵). Ginger et al. (1971) has found that antibiotics are able to suppress the conversion of orally administered prontosil to sulfanilamide in the rat. Hence, concomitant intake of prontosil and antibiotics could affect the pharmacokinetics of prontosil and thus its bioavailability. Also, intraperitoneally injected prontosil undergoes gut bacterial metabolism since it can be transported into the gastrointestinal tract by the enterohepatic circulation⁵⁶. Neoprontosil, a high polar antibacterial drug, is also reduced by gut bacterial enzymes to the pharmacologically active metabolite sulfanilamide. However as a high polar drug, neoprontosil is not easily absorbed in the intestine and a large portion is excreted by bile, unchanged⁵⁶.

Sulfasalazine used in the treatment of inflammatory bowel disease belongs also into same group of sulfonamide drugs. In the colon, the azo bond of sulfasalazine is reduced by bacterial azoreductases forming two metabolites – 5-aminosalicylic acid (mesalazine) and sulfapyridine^{57,58}. On the other hand, unchanged sulfasalazine was found in the caecum and faeces of antibiotic-treated

Fig. 1. Azo reduction of prontosil is one example, where intestinal bacteria convert inactive therapeutics into their pharmacologically active form. Bacterial azoreductases present in the distal gut cleave the N-N double bond and produce active metabolite sulfanilamide.

or with germ-free rats. A metabolite of sulfasalazine, sulfapyridine, is absorbed in the colon and could be reason of side effects of sulfasalazine in some patients, such as nausea, skin rash, headache, dizziness and loss of appetite⁵⁹. The synthesis of **balsalazide** from 4-aminobenzoyl-balanine and salicylic acid was an effort to obviate the side effects of sulfasalazine. Gut microbiota has been shown to metabolize balsalazide to 5-aminosalicylic acid⁶⁰. Another sulfasalazine analog, **olsalazine**, is converted into two 5-aminosalicylic acid molecules by azoreductase-containing bacteria⁶¹.

Nitro reduction

Nitro reductases are the next commonly produced gut bacterial enzymes involved in the metabolism of drugs. Orally administered drugs with a nitro group are reduced to amino products via nitroso and hydroxylamino intermediates⁶². A hypnotic, sedative and anticonvulsant drug of the benzodiazepine class, **nitrazepam** undergoes nitro reduction catalyzed by the gut bacterial enzymes (Fig. 2) (ref.⁶³). In this case, the bacterial metabolic activation does not produce pharmacologically active metabolite but products with teratogenic effects. The gastrointestinal tract is the primary site for nitro reduction of nitrazepam to 7-aminonitrazepam, followed by acetylation in the liver to 7-acetylamino nitrazepam, which is teratogenic in rats and mice⁶⁴. This reductive metabolism of nitrazepam occurs with less intensity in rat liver too⁶⁵. Nitro reduction is also involved in the bacterial modification of the next hypnotic, sedative anticonvulsant drug, **clonazepam**. The

Fig. 2. Nitro reduction of nitrazepam catalyzed by the gut bacterial enzymes produce teratogenic metabolite, 7-Aminonitrazepam. It is an example of clinically undesirable side effect caused by the gut microbiota.

participation of the gut microbiota in this metabolism has been demonstrated by studies on germ-free and ex-germ-free rats⁶⁶.

Misonidazole is a 2-nitroimidazole derivative, which is an effective radiosensitizer used in radiation therapy to cause normally resistant hypoxic tumor cells to become sensitive to the treatment. The metabolite of nitro reduction, 1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol, was found in the excreta of conventional rats in comparison with germ-free rats where the metabolite was not detected. One in vitro study showed that misonidazole is metabolized by gut microbiota into its amino derivative⁶⁷.

Sulfoxide reduction

Sulfinpyrazone is a uricosuric medication used to treat thromboembolic disorders. In the light of experiments on healthy volunteers and ileostomy patients receiving a single oral dose of sulfinpyrazone, the intestinal microbiota seems to be only site of sulfinpyrazone reduction in man⁶⁸. The same study also reported the participation of gut microbiota in the formation of sulfinpyrazone in rabbits in vitro and in vivo⁶⁸. **Sulindac**, also containing the sulfinyl functional group, is a non-steroidal anti-inflammatory drug and is used to treat acute and chronic inflammatory conditions. Strong et al. showed that sulindac is reduced by gut microbiota to sulindac sulfide in man and also in vivo and in vitro in rabbits⁶⁸.

Omeprazole is a derivative of benzimidazole used to treat gastroesophageal reflux disease, peptic ulcer and as a prevention of upper gastrointestinal bleeding. Omeprazole inhibits H⁺/K⁺-ATPase in the parietal cell, suppressing gastric acid secretion. The pharmacokinetics of omeprazole is influenced by gut bacteria, which reduce omeprazole to sulfide metabolites. This reductive metabolism has only been reported under in vitro conditions. Orally administered omeprazole is well absorbed and does not reach the distal gut. Thus bacterial enzymes are unlikely to be involved in this metabolism⁶⁹.

N-oxide reduction

Ranitidine and **Nizatidine** is a histamine H₂ receptor antagonist that inhibits stomach acid production commonly used in the treatment of peptic ulcer and gastroesophageal reflux disease. Using a batch culture fermentation system simulating the colon condition, ranitidine was shown to undergo cleavage on an N-oxide bond causing decreased concentration of the drug⁷⁰. This

Fig. 3. Metabolism of nabumetone (adapted from Nobilis et al., 2013⁷⁸). Nabumetone is *in vitro* reduced by commensal intestinal bacteria into pharmacologically inactive metabolite 4-(6-methoxy-2-naphthyl)-butan-2-ol (reduced nabumetone). These results indicate that gut microbiota may cause lower absorption of nabumetone and thus, lower its bioavailability.

Metabolites of nabumetone: Nabumetone: 4-(6-methoxy-2-naphthyl)-2-butanone ($C_{15}H_{16}O_2$); 6-HNA: 6-hydroxy-2-naphthylacetic acid ($C_{12}H_{10}O_3$); 6-MNA: 6-methoxy-2-naphthylacetic acid ($C_{13}H_{12}O_3$); 6-HO-nb-OH: 4-(6-hydroxy-2-naphthyl)-butan-2-ol ($C_{14}H_{16}O_2$); 6-HO-nb=O: 4-(6-hydroxy-2-naphthyl)-butan-2-one ($C_{14}H_{14}O_2$); 3-HO-nabumetone: 4-(6-methoxy-2-naphthyl)-3-hydroxybutan-2-one ($C_{15}H_{16}O_3$); 6-MeO-nb-OH: 4-(6-methoxy-2-naphthyl)-butan-2-ol ($C_{15}H_{18}O_2$); reduced 3-HO-nabumetone: 4-(6-methoxy-2-naphthyl)-butan-2,3-diol ($C_{15}H_{14}O_3$); O-desmethyl 3-OH-nabumetone: 4-(6-hydroxy-2-naphthyl)-3-hydroxybutan-2-one ($C_{14}H_{14}O_3$); reduced O-desmethyl 3-OH-nabumetone: 4-(6-hydroxy-2-naphthyl)-butan-2,3-diol ($C_{14}H_{12}O_3$).

metabolic modification seems to be partly responsible for the impaired absorption of ranitidine from the colon⁷¹. In a subsequent study, nizatidine was found to be metabolized via cleavage on an N-oxide bond in the presence of colonic bacteria to hydroxyiminonizatidine, while cimetidine and famotidine did not undergo such bacterial metabolism⁷². Antibiotics such as rifampicin were found to decrease the absorption of ranitidine in the gastrointestinal tract⁷³.

Other reductions

Digoxin is a cardiac glycoside used in the treatment of an atrial fibrillation, atrial flutter and sometimes heart failure that cannot be controlled by other medication. A primary mechanism of action of digoxin is inhibition of the Na^+/K^+ ATPase, mainly in the myocardium. The absorption and bioavailability of oral digoxin was a topic of much research 40 years ago⁷⁴. In the human body, digoxin may be converted to the inactive metabolites – dihydrodigoxin and dihydrodigoxigenin. These reduced derivatives bind poorly to the Na^+/K^+ ATPase of cardiac cells and have lower cardiac activity. It has been shown that colonic bacteria are mainly responsible for the formation of these two pharmacologically inactive metabolites⁷⁵.

When the digoxin was given intravenously, the smaller amount of reductive metabolites was found. Lindenbaum et al. (1981) also suggest that the presence of reduced metabolites depends on the individual composition of the intestinal microbiome, since only 10% of patients on prolonged digoxin treatment excluded reduced derivatives of digoxin. The administration of erythromycin and tetracycline suppresses the reduction of digoxin *in vitro* and *in vivo*⁷⁵. Reduction of digoxin to dihydrodigoxin and dihydrodigoxigenin is an example of the pharmacological inactivation of drug by gut microbiota in man⁷⁶.

Zonisamide is a sulfonamide anticonvulsant used to treat epilepsy. By reduction of the benzisoxazole ring, zonisamide is primarily converted to 2-sulfamoylacetylphenol *in vitro*. One *in vivo* experiment on rats showed, that the intestinal microbiota are mainly involved in this modification of zonisamide⁷⁷. Experiments on caecal fluids from rats, mice, hamsters, rabbits, and guinea-pigs also reported the reductive metabolism forming 2-sulfamoylacetylphenol. In line with these results, one experiment with concomitantly administered antibiotics and zonisamide in rats, showed that the antibiotics significantly inhibited the urinary and faecal excretion of 2-sulfamoylacetylphenol, while re-contamination of the

Fig. 4. Chromatographic separation of nabumetone and its metabolites after incubation with the commensal bacteria *E. coli* ATCC 25922.

antibiotic-treated rats with microbiota restored the excretion⁷⁷.

Nabumetone, (4-(6-methoxy-2-naphthyl)-2-butanone), is a widely used as a non-acidic, non-steroidal anti-inflammatory prodrug. After oral administration, nabumetone is converted by oxidative cleavage of its side chain to active metabolite 6-methoxy-2-naphthylacetic acid (6-MNA), a very strong COX-2 inhibitor⁷⁸. Nabumetone is used mainly for the management of pain and inflammation in patients with osteoarthritis and rheumatoid arthritis⁷⁹. The metabolism of nabumetone is well known. Recently, new metabolites of I. phase biotransformation were identified (Fig. 3) (ref.⁷⁸). When nabumetone was incubated with live bacterial suspension of commensal *E. coli* ATCC 25922, a reduced pharmacologically inactive metabolite reduced nabumetone was detected (Fig. 4) (unpublished data). These data suggest that gut bacteria are involved in the metabolism of nabumetone and may cause lower absorption of nabumetone and thus, lower bioavailability of the drug. On the basis of these data, a broad spectrum antibiotic, imipenem, was given to rats to evaluate possible effects of antibiotic on the pharmacokinetics of nabumetone. The pharmacokinetics of the main metabolite of nabumetone in the rat plasma, however, was not significantly influenced⁸⁰.

Metronidazole, a 5-nitroimidazole derivative, is used as an antibiotic and antiprotozoal drug. In the liver, metronidazole is metabolized by side-chain oxidation or glucuronidation to form more polar metabolites. Metronidazole can

also undergo reductive modification, when enzymes of anaerobic bacteria disrupt the imidazole ring and produce reduced metabolites - N-(2-hydroxyethyl)-oxamic acid and acetamide⁸¹. Involvement of the gut bacteria in reductive metabolism of metronidazole has been demonstrated in experiments with germ-free rats, since the metabolites N-(2-hydroxyethyl)-oxamic acid and acetamide were only found in excreta of conventional rats and not in the urine or faeces of germ-free rats^{81,82}. One, in vitro study with *C. perfringens* or rat caecal contents also confirmed the formation of these metabolites⁸². N-(2-hydroxyethyl)-oxamic acid and acetamide were detected in small amounts in the urine of patients treated with metronidazole⁸³. According to four randomized clinical trials, coadministration of mesazalamin was found to have no effect on the pharmacokinetics of metronidazole⁸⁴.

Hydrolysis

The metabolism of **sorivudine** is a good example of the importance of studying the metabolites of orally administered drug and possible changes in their pharmacological activity or even toxicity. The stability of drugs in the intestinal lumen and potential involvement of the gut bacteria on the metabolism should not be overlooked. Sorivudine is a thymine analogue used as an antiviral drug released onto the Japanese market in 1993. The antiviral effect of sorivudine is due to competitive inhibition of viral DNA polymerase. However, eighteen acute deaths of patients were caused by coadministration of sorivudine with oral

Fig. 5. *Bacteroidetes* sp. are responsible for lethal toxicity of sorivudine when concomitantly administered with 5-fluorouracil. Sorivudine metabolite of bacterial metabolism, (E)-5-(2-bromovinyl) uracil causes toxic level of anticancer drug 5-fluorouracil⁸⁵.

5-fluorouracil prodrugs, an anticancer drug. Okuda et al. (1998) investigated the exact mechanism of this toxicity and found that sorivudine metabolite produced by bacterial fermentation was responsible for the lethal effect (Fig. 5)⁸⁵. The metabolite (E)-5-(2-bromovinyl) uracil, produced from sorivudine by gut microbiota – mainly *Bacteroidetes* species, can inhibit the metabolism of 5-fluorouracil and cause toxic levels of the drug⁸⁶. Further studies with germ-free rats indicated that for the formation of (E)-5-(2-bromovinyl) uracil, enterobacteria are mainly responsible⁸⁷. Shortly after the deaths of patients, sorivudine was withdrawn from the market.

Lactulose, the keto analogue of lactose (4-(β-D-galactopyranosyl)- D-fructose), is a non-absorbable sugar used to treat constipation and hepatic encephalopathy. The enzymes of the intestinal bacteria (*Lactobacillus*, *Bacteroides*, *E. coli*) catalyze the hydrolysis of lactulose into fructose and galactose followed by conversion to lactic and acetic acids, which lower the pH within the intestine⁸⁸. Lower pH is favorable for protonization of ammonia and other amines, which can then be eliminated in faeces. This is the mechanism of the laxative effect of lactulose.

Dehydroxylation

L-DOPA, L-3,4-dihydroxyphenylalanine, is used in the clinical treatment of Parkinson's disease caused by dopamine depletion in the substantia nigra. Orally administered L-dopa undergoes decarboxylation in the central nervous system and increases the level of dopamine. However, the principal site for L-dopa decarboxylation seems to be the gastrointestinal tract⁸⁹. The products of decarboxylation are m-tyramine and m-hydroxyphenylacetic acid, which were detected in the urine of conventional rats but not in pathogen free animals. One study on rat faecal content confirmed the importance of the gut microbiota in the dehydroxylation of the catechol ring of L-dopa and forming the metabolites⁸⁹.

Deacetylation

Phenacetin was one of the first synthetic antipyretic drugs on the market introduced in 1887 and mainly used as an analgesic. Orally administered phenacetin is rapidly absorbed from the small intestine and metabolized in the liver to paracetamol (acetaminophen). Only a small amount of phenacetin is deacetylated to *p*-phenetidine. This metabolite was detected when rat caecal content

were incubated with phenacetin under anaerobic conditions⁹⁰. The formation of *p*-phenetidine is relevant to some clinical complications of phenacetin use, such as methemoglobinemia and nephritis⁹⁰.

Acetylation

5-aminosalicylic acid (5-ASA, mesalazine), active metabolite of the prodrugs sulfasalazine, balsalazide and olsalazine, is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis. Small part of orally administered 5-ASA (or its prodrugs) is acetylated by the gut microbiota to N-acetyl-5-aminosalicylic acid. In vitro studies have shown that the intestinal bacteria of rats, guinea pigs, dogs and humans are capable of acetylating both 5-ASA and also sulfapyridine⁹¹. Experiments with faecal suspension of germ-free rats, have shown no acetylating activity. In human faecal suspension, 5-ASA undergoes acetylation under both aerobic and anaerobic conditions as well as by individual bacteria^{92,93}.

Thiazole ring opening

Levamisole, a synthetic imidazothiazole derivative was originally used to treat worm infestations in both humans and animals and the combination with other drugs is used to treat some tumour diseases⁹⁴. Human gut bacteria, mainly *Bacteroidetes* and *Clostridium* spp. metabolize levamisole into three metabolites with opened thiazole ring under anaerobic conditions, levametabol-I, levametabol-II, and levametabol-III. Co-administered with antibiotics, levamisole has a stronger clinical effect due to inhibition of its bacterial metabolism by intestinal bacteria⁹⁴.

Isoxazole scission

Risperidone is an antipsychotic medication exhibiting a very potent serotonin receptor – 5-HT antagonism used to treat schizophrenia, bipolar disorder, and irritability in people with autism. Orally administered risperidone is effectively absorbed and its oral bioavailability is approximately 70% (ref.⁹⁵). Gut microbiota of rats converts risperidone into dihydroxy-risperidone and hydroxyl-keto-risperidone under both aerobic and anaerobic conditions⁹⁶. In the liver, antibiotics decrease the bioavailability of risperidone. No gut bioavailability has been reported⁹⁷.

Deamination

Flucytosine, a fluorinated pyrimidine analogue, is a synthetic antimycotic drug. Vermes et al. found that bacterial enzymes metabolize flucytosine to 5-fluorouracil, since bacterial metabolism was less pronounced in patients treated with antimicrobial agents⁹⁸.

Other reactions

Lovastatin is a statin drug and potent competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (a rate limiting enzyme in cholesterol synthesis), used to lower cholesterol levels to reduce risk of cardiovascular disease. Lovastatin is metabolized by human liver to more polar hydroxyl metabolites⁹⁹. It has long been known that lovastatin and related compounds

Table 1. Summary of drugs metabolized by gut microbiota.

Clinical use	Drug	Influence on drugs	Type of microbial metabolism	Bacterial species involved	Ref.
Antibiotics	Prontosil	prodrug activation	Azo reduction	unknown	55, 56
	Neoprontosil	prodrug activation	Azo reduction	unknown	55, 56
	Metronidazole		Reduction	unknown	81, 82
	Chloramphenicol	increase toxicity	Amine formation and hydrolysis	unknown	103
Anti-inflammatory drugs	Sulfasalazine	prodrug activation	Azo reduction	unknown	57-59
	Balsalazide	prodrug activation	Azo reduction	unknown	60
	Olsalazine	prodrug activation	Azo reduction	unknown	61
	Sulfinpyrazone	increased activity	Sulfoxide reduction	unknown	68
	Sulindac	increased activity	Sulfoxide reduction	unknown	68
	Nabumetone	probably lower bioavailability	Reduction	unknown	80
Analgesics	Mesalazine		Acetylation	unknown	91-93
	Acetaminophen	increased toxicity	O-sulfation; C-S cleavage of acetaminophen-3-cysteine	<i>Clostridium difficile</i>	106
	Phenacetin	decreased activity	Deacetylation	unknown	90
Cardiotonics	Digoxin	decreased cardiac activity	Reduction	<i>Eggerthella lenta</i>	75, 76
Anti-psychotics	Risperidone	inducing symptoms of Parkinson's disease	Isoxazole scission or hydroxylation	unknown	96
	L-Dopa	decreased activity	Dehydroxylation	unknown	89
	Zonisamide	affect reduction	Reduction	unknown	77
Antivirotics	Sorivudine	its microbial metabolite can cause lethal toxicity of 5-fluorouracil when co-administered with it.	Hydrolysis	<i>Bacteroides</i>	87, 85
Antifungals	Flucytosine	increased activity or toxicity	Deamination	unknown	98
Hypolipidemics	Lovastatin	altered pharmacokinetics	Hydrolysis	unknown	52, 100-102
Hypnotics	Nitrazepam	inducing teratogenicity	Nitro reduction	<i>Clostridium leptum</i>	63-65
Antiepileptics	Clonazepam		Nitro reduction	unknown	66
Cytostatics	Misonidazole		Nitro reduction	unknown	67
Antiulcerotics	Omeprazole	forming sulfide metabolites	Sulfoxide reduction	unknown	69
	Ranitidine	decreased intestinal absorption and systemic bioavailability	N-oxide reduction	unknown	70
	Nizatidine		N-oxide reduction		72
Anthelmintics	Levamisole	increased activity	Thiazole ring opening	<i>Bacteroides</i> and <i>Clostridium</i> spp.	94
Prebiotics	Lactulose	stimulating the growth of beneficial bacteria	Hydrolysis	unknown	88

can be metabolized by the gut microbiota to a phosphorylated derivative and a number of hydroxylated microbial metabolites¹⁰⁰⁻¹⁰². Lovastatin is a lactone prodrug that is readily hydrolyzed in vivo to form the pharmacologically active 2-hydroxy lovastatic acid. The involvement of gut microbiota in the metabolism of lovastatin to its bioac-

tive metabolite was demonstrated in vitro and in vivo in experiments with rats. Administration of antibiotics has been shown to reduce the bacterial metabolism of lovastatin in the intestine and thus, decreases the bioavailability of the active metabolite, mainly due to antibiotic-mediated inhibition of intestinal bacteria⁵².

Chloramphenicol is an antibiotic with a broad spectrum activity used to treat a number of bacterial infections. Chloramphenicol contains an amide of dichloroacetic acid and a nitrobenzene group which undergoes bacterial metabolism in the gastrointestinal tract. Incubation of chloramphenicol with bacteria commonly found in human faeces has shown that the gut microbiota is able to metabolite chloramphenicol into a several metabolites, including the toxic metabolite *p*-aminophenyl-2-amin-1,3-propanediol related to risk of marrow aplasia¹⁰³. Marrow aplasia has been shown only in a few percent of patients given chloramphenicol orally and microbiota contained a large amount of coliform microorganisms capable of producing toxic metabolites of chloramphenicol¹⁰³.

Acetaminophen (*N*-acetyl-*p*-aminophenol, paracetamol) is one of the most widely used nonprescription medicines in the world for its analgesic and antipyretic properties. The biotransformation and potential toxicity of acetaminophen have been intensively studied and the fate of this drug in the human body is well known^{104,105}. Orally prescribed acetaminophen is mostly modified into two pharmacologically inactive metabolites, glucuronide and sulfate. A minor fraction is oxidized primarily by cytochromes P450 into a reactive metabolite NAPQI (5-10%), which is responsible for acetaminophen induced toxicity. NAPQI is detoxified by binding with glutathione to form acetaminophen glutathione conjugate, which is ultimately eliminated by the urine. The liver, and to a lesser extent the kidney are the major organs involved in the metabolism of acetaminophen but intestinal bacteria are also engaged, since two microbial cometabolites *p*-cresol sulfate and phenylacetylglutamine have been found. The microbial metabolite *p*-cresol undergoes *o*-sulfonation to produce the *p*-cresol sulfate and competes for the binding site of sulfotransferases with acetaminophen leading to increased acetaminophen toxicity¹⁰⁶. In the light of these results, the role of the gut microbiota in the metabolism of drugs and the microbiome profile/composition of an individual should be taken into consideration, even with the conventionally used drugs with very well studied metabolism such as acetaminophen.

The list of all described drugs is summarized in Table 1.

CONCLUSION

On the basis of recent studies, the human gut microbiota can play a major role in the metabolism of xenobiotics and affect the stability and oral bioavailability of drugs. In the last 50 years, more than 30 drugs have been identified as a substrate for intestinal bacteria. Many questions concerning the impact of the gut microbiota on drug metabolism, however, remain unanswered or answered only partially, namely (i) what are the molecular mechanisms and which bacterial species are involved? what is the impact of host genotype and environmental factors on the composition and function of the gut microbiota, (iii) to what extent is the composition of the intestinal microbiome stable, transmissible, and resilient to perturbation?

(iv) Has a past exposure to a given drug any impact on future microbial response, and, if so for how long? Answering such questions should be an integral part of pharmaceutical research and personalised health care.

Acknowledgment: This work was supported by the Czech Science Foundation (grant no. P303/12/0535), by the internal student's grant of the Palacky University (LF_2016_012) and by the Ministry of Health, Czech republic – conceptual development of research organization (FNOI, 00098892).

Author contributions: LJ: manuscript writing; PA, EA: critical review of the manuscript.

Conflict of interest statement: The authors declare there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Turnbaugh PJ, Stintzi A. Human health and disease in a microbial world. *Front Microbiol* 2011;2.
2. Hill MJ. Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev* 1997;6 Suppl 1:S43-5.
3. Viggiano D, Ianiro G, Vanella G, Bibbo S, Bruno G, Simeone G, Mele G. Gut barrier in health and disease: focus on childhood. *Eur Rev Med Pharmacol Sci* 2015;19(6):1077-85.
4. Zhan Y, Chen PJ, Sadler WD, Wang F, Poe S, Nunez G, Eaton KA, Chen GY. Gut microbiota protects against gastrointestinal tumorigenesis caused by epithelial injury. *Cancer Res* 2013;73(24):7199-210.
5. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003;62(1):67-72.
6. Ferreira CM, Vieira AT, Vinolo MA, Oliveira FA, Curi R, Martins Fdos S. The central role of the gut microbiota in chronic inflammatory diseases. *J Immunol Res* 2014;2014:689492.
7. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, Prifti E, Vieira-Silva S, Gudmundsdottir V, Krogh Pedersen H, Arumugam M, Kristiansen K, Voigt AY, Vestergaard H, Hercog R, Igor Costea P, Kultima JR, Li J, Jorgensen T, Levenez F, Dore J, Nielsen HB, Brunak S, Raes J, Hansen T, Wang J, Ehrlich SD, Bork P, Pedersen O. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 2015;528(7581):262-6.
8. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WHW, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;19(5):576-85.
9. Singh V, Yeoh BS, Vijay-Kumar M. Gut microbiome as a novel cardiovascular therapeutic target. *Current Opinion in Pharmacology* 2016;27:8-12.
10. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. *Pharmacol Res* 2013;69(1):52-60.
11. Stojančević M, Bojić G, Al-Salami H, Mikov M. The influence of intestinal tract and probiotics on the fate of orally administered drugs. *Curr Issues Mol Biol* 2013;4(16):2.
12. Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* 2013;152(1):39-50.
13. Shamat MA. The role of the gastrointestinal microflora in the metabolism of drugs. *International journal of pharmaceutics* 1993;97(1):1-13.
14. Mikov M. The metabolism of drugs by the gut flora. *European journal of drug metabolism and pharmacokinetics* 1994;19(3):201-07.
15. Al-Hilal TA, Alam F, Byun Y. Oral drug delivery systems using chemical conjugates or physical complexes. *Adv Drug Deliv Rev* 2013;65(6):845-64.
16. Consortium HMP. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486(7402):207-14.

17. Miller TL, Wolin M. Methanogens in human and animal intestinal tracts. *Systematic and applied microbiology* 1986;7(2):223-29.
18. Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y. RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biol* 2006;4(1):e3.
19. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *science* 2005;307(5717):1915-20.
20. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, Ugarte E, Muñoz-Tamayo R, Paslier DL, Nalin R. Towards the human intestinal microbiota phylogenetic core. *Environmental microbiology* 2009;11(10):2574-84.
21. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OL, Juge N, Avershina E, Rudi K, Narbad A, Jenmalm MC. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial ecology in health and disease* 2015;26.
22. Biasucci G, Rubini M, Riboni S, Morelli L, Bessi E, Retetangos C. Mode of delivery affects the bacterial community in the newborn gut. *Early Human Development* 2010;86(1, Supplement):13-15.
23. Tojo R, Suarez A, Clemente MG, de los Reyes-Gavilan CG, Margolles A, Gueimonde M, Ruas-Madiedo P. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World J Gastroenterol* 2014;20(41):15163-76.
24. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Dore J, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature* 2011;473(7346):174-80.
25. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences* 2011;108(Supplement 1):4586-91.
26. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences* 2011;108(Supplement 1):4554-61.
27. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012;488(7410):178-84.
28. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, Guigo R, Knight R, Guarner F. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. *Genome research* 2010;20(10):1411-19.
29. Shashkova T, Popenko A, Tyakht A, Peskov K, Kosinsky Y, Bogolubsky L, Raigorodskii A, Ischenko D, Alexeev D, Govorun V. Agent Based Modeling of Human Gut Microbiome Interactions and Perturbations. *PLoS ONE* 2016;11(2):e0148386.
30. Pérez-Cobas AE, Gosalbes MJ, Friedrichs A, Knecht H, Artacho A, Eismann K, Otto W, Rojo D, Bargiela R, von Bergen M. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut* 2013;62(11):1591-601.
31. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *The ISME journal* 2007;1(1):56-66.
32. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2011;108 Suppl 1:4554-61.
33. De La Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP, Dore J. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J Clin Microbiol* 2005;43(11):5588-92.
34. Knip M, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. *Nat Rev Endocrinol* 2016;12(3):154-67.
35. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005;102(31):11070-5.
36. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma. *Curr Opin Rheumatol* 2015;27(4):373-80.
37. Huang YJ, Boushey HA. The microbiome in asthma. *J Allergy Clin Immunol* 2015;135(1):25-30.
38. Legatzki A, Rosler B, von Mutius E. Microbiome diversity and asthma and allergy risk. *Curr Allergy Asthma Rep* 2014;14(10):466.
39. O'Toole PW, Jeffery IB. Gut microbiota and aging. *Science* 2015;350(6265):1214-5.
40. Zapata HJ, Quagliarello VJ. The microbiota and microbiome in aging: potential implications in health and age-related diseases. *J Am Geriatr Soc* 2015;63(4):776-81.
41. Kelly JR, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbiota axis: challenges for translation in psychiatry. *Ann Epidemiol* 2016.
42. Mangiola F, Ianiro G, Franceschi F, Fagioli S, Gasbarrini G, Gasbarrini A. Gut microbiota in autism and mood disorders. *World J Gastroenterol* 2016;22(1):361-8.
43. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto J-M, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464(7285):59-65.
44. Scheline RR. Drug metabolism by intestinal microorganisms. *J Pharm Sci* 1968;57(12):2021-37.
45. Scheline RR. Metabolism of foreign compounds by gastrointestinal microorganisms. *Pharmacol Rev* 1973;25(4):451-523.
46. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int J Pharm* 2008;363(1):1-25.
47. Saad R, Rizkallah MR, Aziz RK. Gut Pharmacomicrobiomics: the tip of an iceberg of complex interactions between drugs and gut-associated microbes. *Gut Pathogens* 2012;4(1):1-13.
48. Li H, He J, Jia W. The influence of gut microbiota on drug metabolism and toxicity. *Expert Opin Drug Metab Toxicol* 2016;12(1):31-40.
49. Kang MJ, Kim HG, Kim JS, Oh do G, Um YJ, Seo CS, Han JW, Cho HJ, Kim GH, Jeong TC, Jeong HG. The effect of gut microbiota on drug metabolism. *Expert Opin Drug Metab Toxicol* 2013;9(10):1295-308.
50. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int J Pharm* 2008;363.
51. Saad R, Rizkallah MR, Aziz RK. Gut Pharmacomicrobiomics: the tip of an iceberg of complex interactions between drugs and gut-associated microbes. *Gut Pathog* 2012;4(1):16.
52. Yoo DH, Kim IS, Van Le TK, Jung IH, Yoo HH, Kim DH. Gut microbiota-mediated drug interactions between lovastatin and antibiotics. *Drug Metab Dispos* 2014;42(9):1508-13.
53. Yoo HH, Kim IS, Yoo DH, Kim DH. Effects of orally administered antibiotics on the bioavailability of amlodipine: gut microbiota-mediated drug interaction. *J Hypertens* 2016;34(1):156-62.
54. Fuller AT. Is p-aminobenzenesulphonamide the active agent in prontosil therapy? *Lancet* 1937;1:194-98.
55. Fouts JR, Kamm JJ, Brodie BB. Enzymatic reduction of prontosil and other azo dyes. *J Pharmacol Exp Ther* 1957;120(3):291-300.
56. Gingell R, Bridges JW, Williams RT. The Role of the Gut Flora in the Metabolism of Prontosil and Neoprontosil in the Rat. *Xenobiotica* 1971;1(2):143-56.
57. Das KM, Eastwood MA, McManus JPA, Sircus W. The metabolism of salicylazosulphapyridine in ulcerative colitis: I The relationship between metabolites and the response to treatment in inpatients. *Gut* 1973;14(8):631-36.
58. Peppercorn MA, Goldman P. The role of intestinal bacteria in the metabolism of salicylazosulphapyridine. *J Pharmacol Exp Ther* 1972;181(3):555-62.
59. Peppercorn MA, Goldman P. Distribution studies of salicylazosulphapyridine and its metabolites. *Gastroenterology* 1973;64(2):240-5.
60. Chan RP, Pope DJ, Gilbert AP, Sacra PJ, Baron JH, Lennard-Jones JE. Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide. *Dig Dis Sci* 1983;28(7):609-15.
61. Wadworth AN, Fitton A. Olsalazine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in inflammatory bowel disease. *Drugs* 1991;41(4):647-64.
62. Nambara S, Yamaha T. [Comparison of bacterial and microsomal azo- and nitro-reductases (author's transl)]. *Yakugaku Zasshi* 1975;95(11):1302-6.

63. Takeno S, Sakai T. Involvement of the intestinal microflora in nitrazepam-induced teratogenicity in rats and its relationship to nitroreduction. *Teratology* 1991;44(2):209-14.
64. Takeno S, Hirano Y, Kitamura A, Sakai T. Comparative developmental toxicity and metabolism of nitrazepam in rats and mice. *Toxicology and applied pharmacology* 1993;121(2):233-38.
65. Rafii F, Sutherland JB, Hansen EB, Jr., Cerniglia CE. Reduction of nitrazepam by *Clostridium leptum*, a nitroreductase-producing bacterium isolated from the human intestinal tract. *Clin Infect Dis* 1997;25 Suppl 2:S121-2.
66. Elmer GW, Rimmel RP. Role of the intestinal microflora in clonazepam metabolism in the rat. *Xenobiotica* 1984;14(11):829-40.
67. Koch RL, Beaulieu BB, Jr., Goldman P. Role of the intestinal flora in the metabolism of misonidazole. *Biochem Pharmacol* 1980;29(24):3281-4.
68. Strong HA, Renwick AG, George CF, Liu YF, Hill MJ. The reduction of sulphinpyrazone and sulindac by intestinal bacteria. *Xenobiotica* 1987;17(6):685-96.
69. Watanabe K, Yamashita S, Furuno K, Kawasaki H, Gomita Y. Metabolism of omeprazole by gut flora in rats. *J Pharm Sci* 1995;84(4):516-7.
70. Basit AW, Lacey LF. Colonic metabolism of ranitidine: implications for its delivery and absorption. *Int J Pharm* 2001;227(1-2):157-65.
71. Basit AW, Podczek F, Newton JM, Waddington WA, Ell PJ, Lacey LF. The use of formulation technology to assess regional gastrointestinal drug absorption in humans. *Eur J Pharm Sci* 2004;21(2-3):179-89.
72. Basit AW, Newton JM, Lacey LF. Susceptibility of the H₂-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora. *Int J Pharm* 2002;237(1-2):23-33.
73. Machavaram KK, Gundu J, Yamsani MR. Effect of ketoconazole and rifampicin on the pharmacokinetics of ranitidine in healthy human volunteers: a possible role of P-glycoprotein. *Drug Metabol Drug Interact* 2006;22(1):47-65.
74. Greenblatt DJ, Smith TW, Koch-Weser J. Bioavailability of drugs: the digoxin dilemma. *Clin Pharmacokinet* 1976;1(1):36-51.
75. Lindenbaum J, Rund DG, Butler VP, Jr., Tse-Eng D, Saha JR. Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. *N Engl J Med* 1981;305(14):789-94.
76. Magnusson JO, Bergdahl B, Bogtoft C, Jonsson UE. Metabolism of digoxin and absorption site. *Br J Clin Pharmacol* 1982;14(2):284-5.
77. Kitamura S, Sugihara K, Kuwasako M, Tatsumi K. The role of mammalian intestinal bacteria in the reductive metabolism of zonisamide. *J Pharm Pharmacol* 1997;49(3):253-6.
78. Nobilis M, Mikusek J, Szotakova B, Jirasko R, Holcapek M, Chamseddin C, Jira T, Kucera R, Kunes J, Pour M. Analytical power of LLE-HPLC-PDA-MS/MS in drug metabolism studies: identification of new nabumetone metabolites. *J Pharm Biomed Anal* 2013;80:164-72.
79. Hedner T, Samulesson O, Wahrborg P, Wadenvik H, Ung KA, Ekbohm A. Nabumetone: therapeutic use and safety profile in the management of osteoarthritis and rheumatoid arthritis. *Drugs* 2004;64(20):2315-43; discussion 44-5.
80. Jourova L AP, Matuskova Z, Nobilis M, Vecera R, Tlaskalova-Hogenova H, Kverka M, Kolar M, Anzenbacherova E. Imipenem, a carbapenem type antibiotic, does not alter pharmacokinetics of a model drug nabumetone. *Toxicol Lett* 2015;238:S332.
81. Koch RL, Goldman P. The anaerobic metabolism of metronidazole forms N-(2-hydroxyethyl)-oxamic acid. *J Pharmacol Exp Ther* 1979;208(3):406-10.
82. Koch RL, Chrystal EJ, Beaulieu BB, Jr., Goldman P. Acetamide--a metabolite of metronidazole formed by the intestinal flora. *Biochem Pharmacol* 1979;28(24):3611-5.
83. Koch RL, Beaulieu BB, Jr., Chrystal EJ, Goldman P. A metronidazole metabolite in human urine and its risk. *Science* 1981;211(4480):398-400.
84. Pierce D, Corcoran M, Martin P, Barrett K, Inglis S, Preston P, Thompson TN, Willsie SK. Effect of MMX(R) mesalamine coadministration on the pharmacokinetics of amoxicillin, ciprofloxacin XR, metronidazole, and sulfamethoxazole: results from four randomized clinical trials. *Drug Des Devel Ther* 2014;8:529-43.
85. Okuda H, Ogura K, Kato A, Takubo H, Watabe T. A possible mechanism of eighteen patient deaths caused by interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. *J Pharmacol Exp Ther* 1998;287(2):791-9.
86. Nakayama H, Kinouchi T, Kataoka K, Akimoto S, Matsuda Y, Ohnishi Y. Intestinal anaerobic bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(E)-(2-bromovinyl)uracil that increases the level and toxicity of 5-fluorouracil. *Pharmacogenetics* 1997;7(1):35-43.
87. Ashida N, Ijichi K, Watanabe Y, Machida H. Metabolism of 5'-ether prodrugs of 1-beta-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil in rats. *Biochem Pharmacol* 1993;46(12):2201-7.
88. Elkington SG, Floch MH, Conn HO. Lactulose in the Treatment of Chronic Portal-Systemic Encephalopathy. *New England Journal of Medicine* 1969;281(8):408-12.
89. Goldin BR, Peppercorn MA, Goldman P. Contributions of host and intestinal microflora in the metabolism of L-dopa by the rat. *J Pharmacol Exp Ther* 1973;186(1):160-6.
90. Smith G, Griffiths L. Metabolism of N-acylated and O-alkylated drugs by the intestinal microflora during anaerobic incubation in vitro. *Xenobiotica* 1974;4(8):477-87.
91. Dull BJ, Salata K, Goldman P. Role of the intestinal flora in the acetylation of sulfasalazine metabolites. *Biochem Pharmacol* 1987;36(21):3772-4.
92. van Hogezaand RA, Kennis HM, van Schaik A, Koopman JP, van Hees PA, van Tongeren JH. Bacterial acetylation of 5-aminosalicylic acid in faecal suspensions cultured under aerobic and anaerobic conditions. *Eur J Clin Pharmacol* 1992;43(2):189-92.
93. Delomenie C, Fouix S, Longuevaux S, Brahimi N, Bizet C, Picard B, Denamur E, Dupret JM. Identification and functional characterization of arylamine N-acetyltransferases in eubacteria: evidence for highly selective acetylation of 5-aminosalicylic acid. *J Bacteriol* 2001;183(11):3417-27.
94. Shu YZ, Kingston DG, Van Tassell RL, Wilkins TD. Metabolism of levamisole, an anti-colon cancer drug, by human intestinal bacteria. *Xenobiotica* 1991;21(6):737-50.
95. He H, Richardson JS. A pharmacological, pharmacokinetic and clinical overview of risperidone, a new antipsychotic that blocks serotonin 5-HT₂ and dopamine D₂ receptors. *Int Clin Psychopharmacol* 1995;10(1):19-30.
96. Meuldermans W, Hendrickx J, Mannens G, Lavrijns K, Janssen C, Bracke J, Le Jeune L, Lauwers W, Heykants J. The metabolism and excretion of risperidone after oral administration in rats and dogs. *Drug Metab Dispos* 1994;22(1):129-38.
97. Baciewicz AM, Chrisman CR, Finch CK, Self TH. Update on rifampin, rifabutin, and rifapentine drug interactions. *Curr Med Res Opin* 2013;29(1):1-12.
98. Vermes A, Kuijper EJ, Guchelaar HJ, Dankert J. An in vitro study on the active conversion of flucytosine to fluorouracil by microorganisms in the human intestinal microflora. *Chemotherapy* 2003;49(1-2):17-23.
99. Greenspan MD, Yudkovitz JB, Alberts AW, Argenbright LS, Arison BH, Smith JL. Metabolism of lovastatin by rat and human liver microsomes in vitro. *Drug Metab Dispos* 1988;16(5):678-82.
100. Endo A, Yamashita H, Naoki H, Iwashita T, Mizukawa Y. Microbial phosphorylation of compactin (ML-236B) and related compounds. *J Antibiot (Tokyo)* 1985;38(3):328-32.
101. Serizawa N, Nakagawa K, Tsujita Y, Terahara A, Kuwano H, Tanaka M. 6 alpha-Hydroxy-iso-ML-236B (6 alpha-hydroxy-iso-compactin) and ML-236A, microbial transformation products of ML-236B. *J Antibiot (Tokyo)* 1983;36(7):918-20.
102. Serizawa N, Nakagawa K, Tsujita Y, Terahara A, Kuwano H. 3 alpha-Hydroxy-ML-236B (3 alpha-hydroxycompactin), microbial transformation product of ML-236B (compactin). *J Antibiot (Tokyo)* 1983;36(5):608-10.
103. Holt R. The bacterial degradation of chloramphenicol. *Lancet* 1967;1(7502):1259-60.
104. Prescott LF. Kinetics and metabolism of paracetamol and phenacetin. *Br J Clin Pharmacol* 1980;10 Suppl 2:291S-98S.
105. Lau GS, Critchley JA. The estimation of paracetamol and its major metabolites in both plasma and urine by a single high-performance liquid chromatography assay. *J Pharm Biomed Anal* 1994;12(12):1563-72.
106. Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc Natl Acad Sci U S A* 2009;106(34):14728-33.