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Gut microbiota-mediated drug-antibiotic interactions

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Running title: **Drug-antibiotic interactions**

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ABSTRACT

Xenobiotic metabolism involves the biochemical modification of drugs and phytochemicals in living organisms, including humans and other animals. In the intestine, the gut microbiota catalyzes the conversion of hydrophilic drugs into absorbable, hydrophobic compounds through hydroxyzation and reduction. Drugs and phytochemicals are transformed into bioactive (sulfasalazine, lovastatin, and ginsenoside Rb1), bioinactive (chloramphenicol, ranitidine, and metronidazole), and toxic metabolites (nitrozepam), thus affecting the pharmacokinetics of the original compounds. However, antibiotics suppress the activites of drug-metabolizing enzymes by inhibiting the proliferation of gut microbiota. Antibiotic treatment might influence xenobiotic metabolism more extensively and potently than previously recognized and reduce gut microbiota-mediated transformation of orally administered drugs, thereby altering the systemic concentrations of intact drugs, their metabolites, or both. This review describes the effects of antibiotics on the metabolism of drugs and phytochemicals by the gut microbiota.

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Introduction

Oral administration is arguably the most complex route of drug delivery. Orally administered drugs are absorbed through the epithelial membrane into the blood. The efficiency of this process is dependent on the solubility, stability, and permeability of the drug, as well its metabolism by enzymes secreted by the body and gut microbiota (Al-Hilal et al., 2013; Davis, 2005; Linnernas and Abrahamsson, 2005). Numberous studies have focused on understanding how drug bioavailability due to the solubility, permeability, and stability in the stomach and duodenum affect drug availability. However, the metabolism of drugs by the gut microbiota has been studied in less detail. The ability of gut bacteria to metabolize xenobiotics and endogenous and exogenous compounds is comparable to that of any organ in the body, including the liver (Mikov, 1994; Saad et al., 2012; Sousa et al., 2008).

Xenobiotic metabolism involves the biochemical modification of drugs or phytochemicals that are not normally present in the living organism (Doring and Petzinger, 2014). These processes occur mainly in the liver. However, recent studies have reported that orally administered xenobiotics are metabolized by gut microbial enzymes before being absorbed from the gastrointestinal tract into the blood (Joh and Kim, 2010; Tralau et al., 2014). The metabolic reactions performed by the liver and the gut microbiota are very different: the liver primarily produces hydrophilic metabolites through oxidative and conjugative metabolism, while the gastrointestinal microbiota mainly generates hydrophobic byproducts through reductive and hydrolytic metabolism (Joh and Kim, 2010). Therefore, gut bacterial metabolism affects the absorption of drugs and can alter their pharmacological effects.

The rate and extent of gut bacterial metabolism are influenced by the amount of drug that

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reaches the distal gut, as well as by the composition of the gut microbial community and the particular enzymes produced by the resident bacteria. Most drugs have little contact with the gut microbiota because they are rapidly and completely absorbed in the upper gut. However, some drugs are transformed to active, inactive, or toxic metabolite(s) by the gut microbiota (Jeong et al., 2013; Sousa et al., 2008; Yoo et al., 2014).

Drug stability and intact drug absorption are clinically relevant to the drug's pharmacological effects. Metabolism can render a drug pharmacologically active, inactive, or toxic. For example, azoreductases produced by colonic bacteria metabolize orally administered sulfasalazine to 5-aminosalicylic acid, a metabolite that induces anti-inflammatory effects by inhibiting pro-inflammatory mediators (Hayllar and Bjarnason, 1991; Klotz, 1985; Peppercorn and Goldman, 1976). Therefore, sulfasalazine is used in the treatment of mild-to-moderate ulcerative colitis. However, cotreatment with antibiotics attenuates the pharmacological effect of sulfasalazine by disturbing the gut microbiota and altering the metabolism of gut microbiota.

In light of the importance of drug metabolism by the gut microbiota, this review describes gut microbiota-mediated interactions between antibiotics and drugs or phytochemicals.

Gut microbiota

The gut microbiota of humans and other animals comprises more than a thousand microorganisms (Cho and Blaser, 2012; Lakshminarayanan et al., 2014). Most of these microbes reside in the ileum and colon. Their primary function is to ferment carbohydrates and proteins that are not digested in the upper gut into absorbable energy. Other functions of these bacteria include producing vitamins (B and K), protecting against pathogens,

mediating innate and adaptive immune responses, and metabolizing orally administered natural products and drugs.

The composition of the gut microbiota as well as the residence of specific bacterial species is affected by pH, diet, the use of antibiotics, the presence of digestive enzymes, the redox potential of the tissue and gut transit time (Aguilera et al., 2013; Nord, 1990; Oktyabrsky and Smirnova, 1989; Xu et al., 2014a). Conditions are extremely variable in the gastrointestinal tract, mouth, pharynx, esophagus, stomach, small intestine, and large intestine. For example, regions with a low pH create a harsh environment for bacterial residence and growth and thus often limit species diversity. With respect to the impact of redox potential on the number and species of bacteria that colonize the gut, regions with a lower redox potential favor the growth of bacteria that actively metabolize carbohydrates to short-chain fatty acids (Oktyabrsky and Smirnova, 1989; Xu et al., 2014a). Gastrointestinal transit time is also associated with bacterial growth and metabolism. The mean whole-gut transit time in humans is 70 h with times ranging from 23 to 168 h (Cummings et al., 1992). Although transit times vary between individuals, intestinal fluids typically spend the longest time in the large intestine, rather than in the stomach and small intestine (Tuleu et al., 2002; Varum et al., 2008; Wilding, 2001). Slow colonic transit times increase the production of bacterial metabolites, such that bacterial metabolism in the small intestine is lower than that in the large intestine (Cummings et al., 1979).

In the last century, scientists have detected and identified many species in the human gut microbiota (Savage, 2001). Current estimates for the total number of bacteria that reside in the human gut are as high as 100 trillion (Lakshminarayanan et al., 2014; Ley et al., 2006). For counting and identifying the bacteria present, most conventional methods involve

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diluting of the intestinal fluid samples, incubating of the samples with specific growth media, and then determinating of the number and species of cultured bacteria (Cani, 2013; Marchesi, 2011). Studies using these methods have suggested that at least 400 bacterial species inhabit the human gastrointestinal tract. However, not all bacteria can be cultured in growth media. Recent advances have made it possible to study bacterial populations with culture-independent approaches that use molecular genetic methodologies such as 16S RNA pyrosequencing. Ribosomal RNA gene sequencing methods are ideal for the classification of organisms. Studies using these newer, molecular methods estimate that the human gastrointestinal microbiota comprises over 2000 species. Most species belong to eight dominant phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria*, and *Spirochaetes* (Eckburg et al., 2005; Wang et al., 2005). More than 80% of the species belong to the phyla *Firmicutes* and *Bacteroides*. *Firmicutes*, the most abundant and diverse group, includes clostridia and bacilli. *Bacteroidetes* is also present in high numbers (Eckburg et al., 2005; Wang et al., 2005). However, molecular techniques might overestimate the number of bacterial species in the gut by failing to distinguish between resident and transient microbes.

Metabolism of drugs by the gut microbiota

The liver is a major site of xenobiotic metabolism. Most xenobiotic metabolic processes in the liver convert hydrophobic compounds into hydrophilic products, and thereby facilitate their excretion and detoxification. Conversely, the metabolism of orally administered xenobiotics in the intestine by the gut microbiota transforms hydrophilic compounds into hydrophobic metabolites, allowing these products to be absorbed from the gastrointestinal

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tract into the blood. The activity and toxicity of the transformed hydrophobic metabolites can differ from those of the parent drugs and phytochemicals (Jin et al, 2014; Yoo et al., 2014; Gratz et al., 2013).

Many orally administered hydrophilic drugs are not easily digested in the presence of gastric and pancreatic juices. Therefore, many hydrophilic drugs pass through the upper intestinal tract and reach the lower tract, where numerous bacteria reside (Macfarane and Macfarane, 2004; Mikov, 1994; Pieper and Bertau, 2010). Bacteria then metabolize the drugs to hydrophobic compounds, which exert their pharmacological effects after absorption. Representative examples of xenobiotics and phytochemicals metabolized by the gut microbiota include lovastatin, simvastatin, protosil, digoxin, irinotecan, glycyrrhizin, amygadalin, baicalin, ginsenosides, and genistein.

Antimicrobial drugs and phytochemicals affect bacterial growth and colonization in the gastrointestinal tract. Consequently, they significantly affect bacterial metabolism in the gut. The effect of antibiotics on xenobiotic metabolism is more extensive and potent than previously recognized (Jin et al., 2010; Yoo et al., 2014). Most antibiotics disturb the composition and enzyme activities of the gut microbiota and can suppress gut microbial enzyme activity for more than 3 days. We have previously described the effect of antibiotic treatment on the pharmacokinetics of drugs and phytochemicals (Jin et al., 2010; Yoo et al., 2014) which is supported by the results of several other studies (Saad et al., 2012; Sousa et al., 2008; Shu et al., 1991). In the gut, when antibiotics affect the activity of another drug administered concomitantly, a novel type of drug-drug interaction occurs, distinct from those that occur in the liver. Table 1 lists the drugs and phytochemicals that metabolized by

the gut microbiota in a manner that is altered by the co-administration of antibiotics. This is will discussed in more detail in the following section. Drug-drug interactions involve various processes, including pharmacokinetic and pharmacodynamic interactions. Alterations in drug pharmacokinetics (absorption, distribution, metabolism, and excretion) are generally due to the inhibition or induction of drug metabolizing enzymes, such as cytochrome P450 enzymes or transporters involved in absorption and excretion. Modulation of gut microbial enzyme activity is another possible cause of drug-drug interactions. Drugs (generally antibiotics) that affect the metabolic activities of gut microbes can alter the pharmacokinetics of co-administered drugs that are metabolized by gut microbiota. Even though the effect of the gut microbiota on drug metabolism has been recognized, potential drug-drug interactions that occur via this mechanism have not been considered.

The main sites for xenobiotic metabolism by gut microbiota, the distal small intestine and the large intestine, are inaccessible in living organisms. Consequently, the metabolism of drugs in the intestine cannot be examined directly. To elucidate the effects of antibiotics on the gut microbiota-mediated metabolism of drugs and phytochemicals, in vitro and in vivo methods have been developed, including the following: continuous culture systems; simulations of the human intestinal microbial ecosystem; and gnotobiotic, pseudo-germ-free, and germ-free animal models. None are ideal for mimicking the natural interactions in the guts (Edwards and Parrett, 1999; Sousa et al., 2008).

Drugs metabolized by the gut microbiota

Azo reduction of drugs

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Prontosil: Prontosil, produced in Germany, was the first commercially available antibacterial drug. When analyzed in vitro, prontosil exhibits minimal antibacterial activities. However, when orally administered in a murine model of *Streptococcus pyogenes* systemic infection, prontosil was transformed to sulfanilamide by azoreductases produced by the gut microbiota. This metabolite was found to exhibit potent antibacterial activity. In addition to gut bacteria, the liver and kidney also convert protosil to sulfanilamide (Fig. 1A) (Fouts et al., 1957; Gingell et al., 1971; Gingell and Bridges, 1973). Intraperitoneally injected prontosil, excreted into the intestine via the bile, is metabolized to sulfanilamide by the azoreductases produced by gut bacteria. Treatment with antibiotics suppresses the conversion of orally administered prontosil to sulfanilamide in rats (Gingell et al., 1971).

Neoprontosil: Orally administered neoprontosil, an antibacterial drug that is more polar than prontosil, is not easily absorbed from the intestine. However, after intraperitoneal injection, the drug is excreted via the bile without conversion in the intestine. The gut microbiota converts excreted neoprontosil to the pharmacologically active metabolite sulfanilamide (Gingell et al., 1971). In an in vitro study, rat cecal and fecal homogenates potently transformed neoprontosil to sulfanilamide. Treatment with antibiotics reduced the amount of sulfanilamide excreted in the urine after oral administration of neoprontosil (Gingell et al., 1971).

Sulfasalazine: Sulfasalazine was developed in the 1950s to treat rheumatoid arthritis. Sulfasalazine, a sulfa drug combining sulfapyridine and aminosalicylate with an azo bond, is used for the treatment of ulcerative colitis. Sulfasalazine is barely absorbed by the upper

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intestine, but in the colon, its azo bond is reduced by gut bacteria, releasing 5-aminosalicylic acid (mesalazine; active in the colon) and sulfapyridine (systemically absorbed) (Hayllar and Bjarnason, 1991; Peppercorn and Goldman, 1973 and 1976). Mesalazine is metabolized to acetylated mesalazine (Dull et al., 1987): in the fecal suspensions from rats, dogs, and humans, mesalazine (<5%) is acetylated. However, the fecal suspensions from germ-free rats did not exhibit acetylating activity. In antibiotic-treated rats, the metabolism of sulfasalazine is suppressed in the cecum and feces (Klotz, 1985).

Balsalazide: To overcome the adverse effects of sulfapyridine experienced by some patients (anorexia, nausea, skin rash, blood dyscrasias), balsalazide was synthesized by diazo coupling of salicylic acid with 4-aminobenzoyl-β-alanine instead of the sulfapyridine moiety in sulfasalazine. When orally administered in humans, balsalazide was barely detected in the feces and urine, but 5-aminosalicylic acid was detected (Chan et al., 1983). Thus, the gut microbiota potently metabolizes balsalazide to 5-aminosalicylic acid. However, antibiotic treatment suppresses the bacterial metabolism of balsalazide in humans thus limiting it effectiveness (Chan et al., 1983).

Nitro reduction of drugs

Nitrazepam: Orally administered nitrazepam, a hypnotic, sedative, anticonvulsant, and anxiolytic drug, is metabolized to 7-amino-nitrozepam in rats by the gut microbiota (Fig. 1B) (Rafii et al., 1997; Takeno and Sakai, 1990; Takeno et al., 1993). The metabolite is an active teratogenic substance. Antibiotic treatment reduced nitrazepam-induced teratogenicity in rats relative to that in untreated rats. Studies suggest that a nitroreductase transforms nitrazepam

to a teratogenic metabolite and that gut microbiota are responsible for the reductive metabolism. The reductive metabolism of nitrazepam has been reported to occur in the rat liver. However, reductive metabolism is more potent in rat cecal fluid than in the liver.

Clonazepam: Clonazepam, a hypnotic, sedative, anticonvulsant, and anxiolytic drug, is metabolized to 7-aminoclonazepam. The results of a study using germ-free and ex-germ-free rats support the reductive metabolism of clonazepam by gut microbiota. Similar to the findings for nitrozepam, the reductive metabolism of clonazepam is more potent in the rat gut microbiota than in the tissues (Elmer and Remmel, 1984). Antibiotic treatment inhibits the reduction of clonazepam to 7-aminoclonazepam.

Misonidazole: Misonidazole, a 2-nitroimidazole derivative, is an effective radiosensitizer of hypoxic cells in the treatment of human cancer. When incubated with intestinal microbiota, misonidazole is metabolized to its amino derivative, 1-(2-aminoimidazol-1-yl)- 3-methoxypropan-2-ol, which is further metabolized to release CO_2. The metabolite is detected in the excreta of conventional rats, but not in that of germ-free rats (Koch et al., 1980). Antibiotic treatment inhibits misonidazole transformation and toxicity (Sheldon et al., 1984).

Sulfoxide reduction of drugs

Sulfinpyrazone: Sulfinpyrazone, a uricosuric agent for thromboembolic disorders, is metabolized to sulfinpyrazone sulfide by the gut microbiota of rabbits in vitro and in vivo (Fig. 1C). Metronidazole, but not tetracycline, decreases the extent of sulfinpyrazone

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reduction in rabbits in vivo. The plasma concentration–time curves of healthy volunteers and ileostomy patients who received a single dose were compared, and gut microbiota were found to be the source of sulfinpyrazone reduction in humans (Strong et al., 1987).

Sulindac: Sulindac, an arylalkanoic acid derivative, is a non-steroidal anti-inflammatory drug used to treat rheumatoid arthritis. A pharmacokinetics study in healthy volunteers and ileostomy patients showed that the gut microbiota significantly transforms sulindac sulfide (Strong et al., 1987). The formation of sulfides of sulindac ex vivo is decreased in the feces obtained from patients treated with metronidazole. Sulindac is metabolized to sulindac sulfide by the gut microbiota of rabbits in vitro and in vivo.

N-oxide reduction of drugs

Ranitidine and nizatidine: The in vitro stability of the H2-receptor antagonists, ranitidine, cimetidine, famotidine, and nizatidine in the presence of colonic bacteria has been assessed (Basit et al., 2002). The gut microbiota metabolizes ranitidine and nizatidine to hydroxyiminoranitidine and hydroxyiminonizatidine, respectively, via cleavage of an N-oxide bond (Fig. 1D). However, no such bacterial metabolism has been observed for cimetidine or famotidine (Basit and Lacey, 2001; Basit et al., 2004). Treatment with antibiotics such as rifampicin decreases the absorption of ranitidine by decreasing the percentage of the total dose that disappears in the duodenal, jejunal, and ileal regions of the intestinal loops (Machavaram et al., 2006).

Loperamide oxide: Loperamide oxide is a prodrug of loperamide, which is a widely used,

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effective drug for the symptomatic management of diarrhea. Loperamide oxide is reduced in the gut contents of rats, dogs, and humans, with the most extensive reduction found in cecal contents. In germ-free rats, the cecum shows <1% of the activity found in the small intestine (Lavrijsen et al., 1995). The gut microbiota isolated from rats and dogs reduces loperamide oxide to loperamide under anaerobic conditions, indicating that the microbiota is primarily involved in the reduction. The rate of reduction parallels the cellular uptake of loperamide oxide. The absorption of orally delivered loperamide oxide is lower when administered with cotrimoxazole than when administered loperamide alone (Kamali and Huang, 1996).

Other drugs reductions involving the gut microbita

Digoxin: Orally administered digoxin, a cardiac glycoside clinically used for the treatment of various heart diseases, atrial fibrillation, and atrial flutter, is converted to the inactive metabolites dihydrodigoxin, dihydrodigoxigenin, or both by gut microbiota in some patients (Fig. 1E) (Lindenbaum et al. 1981). Gut microbiome metabolism markedly attenuates the drug's effects because the metabolites bind poorly to the Na^+-K^+-ATPase of cardiac cells. Treatment with the antibiotics erythromycin and tetracycline blocks the reduction of digoxin in vitro and in vivo (Lindenbaum et al., 1981). Further, a study performed in four volunteers showed that the gut microbiota catalyzes the metabolic reaction in the distal small intestine (Magnusson et al., 1982).

Zonisamide: Zonisamide, an anticonvulsant used clinically to treat epilepsy, is metabolized to 2-sulfamoyacetylphenol by gut microbiota in vitro through the reduction of the benzisoxazole ring (Kitamura et al. (1997). Further, cecal fluids from rats, mice, hamsters,

rabbits, and guinea-pigs transform zonisamide to 2-sulfamoyacetylphenol. Treatment with antibiotics significantly inhibits the urinary and fecal excretion of 2-sulfamoylacetylphenol in these animals (Kitamura et al., 1997).

Metronidazole: Metronidazole, a 5-nitroimidazole derivative, is an anti-protozoan and anti-bacterial drug. It is weakly converted to the reduced metabolites N-(2-hydroxyethyl)-oxamic acid and acetamide by rat cecal contents or *Clostridium perfringens*, an anaerobic gut bacterium (Koch and Goldman, 1979; Koch et al., 1979). When conventional and germ-free rats were treated with metronidazole, N-(2-hydroxyethyl)-oxamic acid and acetamide were detected only in conventional rats (Koch et al., 1979; Koch and Goldman, 1979). The metabolites have also been found in the urine of human patients treated with metronidazole (Koch et al., 1981). Mesalamine treatment does not affect the pharmacokinetics of metronidazole (Pierce et al., 2014).

Deglycosylation of drugs

Lactulose: The pharmacological efficacy of lactulose, the keto analogue of lactose (4-(β-D-galactopyranosyl)-D-fructose), is dependent on gut bacterial metabolism. It is metabolized to fructose and galactose by several kinds of gut bacteria (*Lactobacillus, Bacteroides,* and *E. coli*), and the metabolites are further transformed to lactic and acetic acids. The acidic products lower the pH in the intestinal fluid, inhibiting the absorption of ammonia and amines into the blood and accelerating the excretion of protonated amines into the feces (Elkington et al., 1969). Combination treatment with neomycin and lactulose significantly reduces the blood ammonia concentration in pigs (van Berlo et al., 1988).

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Glucuronide-conjugated drugs: Orally, intravenously, intramuscularly, or intraperitoneally administered drugs are primarily metabolized to hydrophilic metabolites via sulfation, glucuronidation, oxidation in tissues such as the liver. They are then partially excreted in the intestine via the bile. However, the gut microbiota then converts the excreted metabolites into deconjugated compounds, which are reabsorbed into the blood (Abe et al., 1990; Al-Hilal et al., 2013). Drugs such as acetaminophen, indomethacin, irinotecan, morphine, and digoxin are often conjugated as glucuronides and sulfates and are excreted in the bile (Orme and Back, 1990; Peppercorn and Goldman, 1976; Simon and Gorbach, 1984). Mucosal and bacteria β-glucuronidases, sulfatases, or both in the intestine catalyze deconjugation reactions, the prerequisite step for reabsorption. Therefore, the gut microbiota plays an important role in the enterohepatic circulation of some drugs. For example, the prodrug irinotecan is hydrolyzed by a carboxylesterase in the liver to form the active metabolite SN-38, which exhibits antitumor activity (Yamamoto et al., 2008). Further, SN-38 is mainly metabolized by UDP glucuronosyltransferase 1A1 in the liver to form inactive SN-38G (detoxification), which is excreted into the intestine via the bile duct and then deconjugated to SN-38 by the β-glucuronidases of the gut microbiota. SN-38 causes diarrhea. Therefore, modulation of SN-38-induced diarrhea in humans by co-administration of the poorly absorbed aminoglycoside antibiotic neomycin could be advantageous (Kehrer et al., 2001).

Desulfation of drugs

Sodium picosulfate (laxoberon) is widely used for the treatment of acute and chronic constipation. After oral ingestion, sodium picosulfate reaches the colon without significant

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absorption, where it is metabolized to the free diphenol [4,4'-(pyridin-2-ylmethanediyl) diphenol] by the gut microbiota (arylsulfate sulfotransferase of *Eubacterium rectale*). The free diphenol has a laxative effect (Kim and Kobashi, 1986; Kim et al., 1992; Kobashi et al., 1986). Time (6 - 12 h) is needed for the gut microbiota to metabolize laxoberon to the free phenol. Treatment with antibiotics inhibits the transformation of laxoberon.

Dehydroxylation of drugs

L-Dopa is used to treat dopamine depletion within the central nervous system in Parkinson's disease. Orally administered L-dopa is thought to undergo decarboxylation within the central nervous system and exert its effect by increasing dopamine levels. However, most of the L-dopa is dehyroxylated to tyramine or m-hydroxyphenylacetic acid in the gut microbiota, not in the central nervous system (Fig. 1F) (Goldin et al., 1973; Peppercorn and Goldman, 1976). Treatment with antibiotics such as vancomycin inhibits the dehydroxylation of bile acid by the gut microbiota.

Deamination of drugs

Flucytosine, which exhibits anti-fungal properties, is metabolized in vitro to 5-fluorouracil by microorganisms isolated from the gut microbiota (Fig. 1G) (Harris et al., 1986; Vermes et al. 2003). Consistent with this, when flucytosine was given to patients receiving antimicrobial agents, the level of 5-fluorouracil production decreased (Vermes et al., 2003). Thus, antimicrobial agents may reduce the anti-fungal effect of flucytosine.

Ring fissuring of drugs

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Thiazole ring (levamisole): Levamisole, an anthelmintic drug used in veterinary and human medicine, has been used to treat colon cancer (Shu et al., 1991). Levamisole is metabolized to three thiazole ring-opened metabolites, namely, levametabol-I, levametabol-II, and levametabol-III, under anaerobic conditions by human gut bacteria, such as *Bacteroides* spp. and *Clostridium* spp. (Fig. 1H) (Shu et al., 1991). Combined therapy with tetracycline and levamisole has a stronger biological effect than levamisole alone because the antibiotic inhibits the metabolism by gut bacteria.

Isoxazole ring (risperidone): Risperidone, an antipsychotic drug, is a potent antagonist of serotonin-5HT2 and dopamine-D2. Under aerobic and anaerobic conditions in vitro and in vivo, the gut microbiota of rats metabolizes risperidone to dihydroxy-risperidone and hydroxy-keto-risperidone via scission of isoxazole (Fig. I) (Meuldermans et al., 1994). Antibiotics such as rifampin inhibit the bioavailability of risperidone in the liver, but the bioavailability in the gut was not reported (Baciewicz et al., 2013).

Tetrahydro-oxopyrane ring (lovastatin and simvastatin): The gut microbiota metabolizes lovastatin to 2-hydroxy lovastatic acid in vitro and in vivo (rats). Antibiotic treatment reduces the bacterial metabolism of lovastatin in the intestine (Yoo et al., 2014) and thus, inhibits the absorption of 2-hydroxy lovastatic acid, an active form of lovastatin (Fig. 1J). Simvastatin is metabolized to 2-hydroxy simvastatin acid through the hydrolytic cleavage of methylbutanoic acid from the backbone (Kantola et al., 1998; Methaneethorn et al., 2014). These findings suggest that the gut microbiota metabolizes lovastatin and simvastatin to an active form of lovastatin and that co-treatment with antibiotics suppresses the

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pharmacological effects of lovastatin and simvastatin.

Phytochemicals metabolized by the gut microbiota

Phytochemicals are chemical compounds that occur naturally in plants. As many as 4,000 different phytochemicals have the potential to affect diseases such as cancer, chronic inflammation, diabetes, and stroke. Many of these phytochemicals are hydrophilic. Therefore, when orally administered to humans and other animals, their bioavailability is generally low (<10%) (Bonifacio et al., 2014; Saad et al., 2012). The gut microbiota can metabolize orally administered phytochemicals to bioactive, toxic, or inactive hydrophobic compounds, as with the hydrophilic drugs described above. Once absorbed into the blood, these hydrophobic metabolites can then exert their pharmacological effects.

Reduction of phytochemicals

Isoflavones: Isoflavones have been reported to ameliorate breast and prostate cancer, osteoporosis, and obesity (Jungbauer and Medjakovic, 2014; Vitale et al., 2013). Their estrogenic effects might be due to the ability of gut microbiota to produce equol from isoflovones (Sepehr et al., 2009; Setchell and Clerici, 2010; Yokoyama and Suzuki, 2008). Intestinal bacteria such as *Adlercreutzia equolifaciens*, *Slackia isoflavoniconvertens*, *Slackia equolifaciens*, and *Lactococcus garvieae* metabolize the isoflavones daidzein, and genistein are metabolized to 5-hydroxy-equol in humans and other animals. When daidzein and genistein which were orally administered to male and female rats harboring a simplified human microbiota without or with *S. isoflavoniconvertens*, the metabolites equol and 5-hydroxy-equol were found in the intestinal contents, feces, and urine. Reductases produced

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by gut microbiota, particularly *S. isoflavoniconvertens,* convert daidzein and genistein to 5-hydroxy-equol via hydroxyisoflavanone or hydroxyisoflavan. Some antibiotics inhibit the conversion of glycosides to aglycones or equol in humans and monkeys (Blair et al., 2003; Halm et al., 2008).

Sennosides: The gut microbiota converts sennosides A and B, the main constituents of senna and rhubarb, to active compounds in the distal intestine. Reductase(s) and 3- β-D-glucosidase(s) of the gut microbiota convert sennosides to rheinanthrone, a purgative compound, via 8-glucosyl-rheinanthrone or sennidin monoglucosides (Hattori et al., 1982 and 1988; Kobashi et al., 1980). Treatment with antibiotics such as chloramphenicol, streptomycin, and rifampicin inhibits the biotransformation of sennosides by inhibiting metabolic enzyme production (Yang et al., 1996). These findings suggest that hydrophilic sennosides are not absorbed in the upper intestine, but reach the distal intestine, where they are converted to rheinanthrone, which has a purgative effect (Hattori et al., 1982).

Deglycosylation of phytochemicals

Glycyrrhizin: Glycyrrhizin, a sweet-tasting compound in the root of *Glycyrrhiza glabra* and *Glycyrrhiza uralensis*, is used in Japan for the treatment with hepatitis C. The gut microbiota metabolizes orally administered glycyrrhizin is metabolized to 18β-glycyrrhetinic acid (>95%) in vitro and in vivo (Hattori et al., 1983; Kim et al., 2000; Takeda et al., 1996). When orally ingested, the parent compound is not detectable in the plasma, whereas 18β-glycyrrhetic acid is detected, although not in the plasma of germ-free rats. These findings suggest that the gut microbiota completely converts glycyrrhizin to 18β-glycyrrhetic acid

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and that the latter is absorbed from the intestine. Treatment with antibiotics such as amoxicillin and metronidazole suppresses the conversion of glycyrrhizin to the aglycone (He et al., 2001).

Ginsenoside Rb1: Ginsenoside Rb1 is the main constituent of *Panax ginseng*, used as a traditional remedy for cancer, inflammation, stress, and ageing (Choi, 2008). The gut microbiota metabolizes orally administered ginsenoside Rb1 to bioactive compounds such as 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) (Akao et al., 1998). Treatment with antibiotics inhibits the metabolism of ginsenoside Rb1 to compound K in vivo (Joh et al., 2011; Xu et al., 2014b). The compound K-forming activity in individuals is proportional to the area under the curve of compound K when ginseng is orally administered to humans (Lee et al., 2009). Taxonomy-based analysis of the human gut microbiota with 16S rRNA gene pyrosequencing showed that the population of *Oscillibacter* spp, *Ruminococcus* spp, *Holdemania* spp, and *Sutterella* spp is related to the compound K-forming activity of the fecal microbiota (Kim et al., 2013). The pharmacological effects of compound K, which includes antidiabetic, anti-inflammatory, and hepatoprotective effects, are more potent than those of the parent ginsenosides Rb1, Rb2, and Rc. Thus, the pharmacological effects of ginseng are dependent on the individual's gut microbiota.

Puerarin and daidzin: Puerarin, an isoflavone C-glycoside, and daidzin, an isoflavone O-glycoside, exhibit anticancer, antiobesity, and estrogenic effects (Jungbauer and Medjakovic, 2014; Lin et al., 2009; Michihar et al., 2012; Vitale et al., 2013). When puerarin or daidzin is incubated with human intestinal microbiota in vitro, two metabolites, daidzein and calycosin, are produced. Puerarin and daidzin are converted to daidzein by C-glucosidases and O-

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glucosidases, respectively (Kim et al., 1998b), and then to calycosin by methyl-transferase and hydroxylase (Kim et al., 1998b; Yasuda and Ohsawa, 1998). Additionally, orally administered puerarin and daidzin are metabolized to equol (Fig. 2A) (Setchell and Clerici, 2010). The metabolites are then absorbed from the intestine into the blood. The biological effects of the metabolites calycosin and daidzein are superior to those of puerarin and daidzin. Antibiotic treatment inhibits the metabolism of isoflavone glycosides to the respective aglycones (Franke et al., 2004).

Hesperidin: Flavonoid rhamnoglycosides including hesperidin, naringin, poncirin, and rutin, are biologically active flavanone glycosides contained in traditional Chinese medicine. The glycosides are metabolized to the respective aglycones and then degraded to phenolic acids such phenylacetic acid and hydroxyphenyl acetic acid (Kim et al., 1998a). Antibiotic treatment inhibits the metabolism of hesperidin to hesperetin in rats and suppresses gut bacterial glycosidase activities (Jin et al., 2010).

Hydroxylation and methylation of phytochemicals

Daidzein: In addition to equal, daidzein is also transformed to calycosin by the gut microbiota, suggesting that the gut microbiota produces aromatic hydroxylase and O-methyltransferase (Kim et al., 1998b; Yasuda and Ohsawa, 1998). Orally administered baicalin is also transformed to oroxylin A via baicalin in vitro and in vivo (Abe et al., 1990; Trinh et al., 2010). The process involves aromatic hydroxylase and O-methyltransferase produced by the gut microbiota. Treatment with antibiotics suppresses the transformation of daidzein in vitro and in vivo (Halm et al., 2008; Sutherland et al., 2012).

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Flavonoid C-ring fissuring of phytochemicals

Flavonoid glycosides such as rutin, hesperidin, naringin, baicalin, wognoside, and poncirin are metabolized to phenolic acids via aglycones by C-ring cleavage and deglycosylating enzymes produced by the gut microbiota of humans and mice (Fig. 2B) (Kim et al., 1988a). (+)-Catechin, (-)-epicatechin, and anthocyanidins are transformed to phenolic acids through a similar process (Cardona et al., 2013; Kim et al., 1998a; Selma et al., 2009). Orally administered flavonoids are transformed to phenolic acids in rats. The metabolites are absorbed into the blood and excreted into the urine. Treatment with antibiotics reduces the levels of C-ring cleaved metabolites excreted into the urine of rats. The phenolic metabolites produced from the orally administered flavonoids might exhibit aspirin-like pharmacological effects. Antibiotic treatment inhibits the biotransformation of flavonoids to the aglycones that mediate these effects (Jin et al, 2010; Trinh et al., 2010).

Summary

Orally administered drugs and food constituents inevitably encounter the microbiota in the gastrointestinal tract. Some of these drugs and phytochemicals are metabolized by the microbiota before they can be absorbed into the blood. Gut microbial metabolism catalyzes the conversion of hydrophilic drugs such as sulfasalazine, digoxin, lovastatin, and laxoberon to hydrophobic compounds via hydroxylation and reduction. This metabolism is distinct from liver metabolism, which catalyzes the conversion of hydrophobic drugs into hydrophilic products through oxidation and glucuronide/sulfate conjugation. Therefore, gut microbiota-mediated metabolism promotes pharmacological effects and enhances absorption,

whereas liver metabolism promotes detoxification. The composition of the gut microbiota and the associated enzyme activities fluctuate significantly in response to environmental factors such as diet, stress, and the presence of antibiotics. Antibiotics, in particular, can dramatically affect drug metabolism by the gut microbiota. For example, when administered together with drugs such as lovastatin, sulfasalazine, and nitrozepam, antibiotics suppress drug-metabolizing enzyme activities by inhibiting the proliferation of the gut microbiota. The effect of antibiotic treatment on in vivo xenobiotic metabolism may be more extensive and potent than previously recognized. Antibiotic treatment may reduce the gut microbial transformation of orally administered drugs in the gut and thereby affect the pharmacologic response by altering the systemic concentrations of the intact drug. Therefore, when orally administered drugs are used with antibiotics, their pharmacological effects should be carefully monitored.

Authorship contributions

Participated in research design, performed data analysis, and wrote the manuscript: D.H. Kim.

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Footnotes

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Figure Legends

Fig. 1. Metabolic reactions of drugs by gut microbiota: (A) protonsil; (B) nitrozepam; (C) sulfinpyrazone; (D) ranitidine; (E) digoxin; (F) L-dopa; (G) flucytosine; (H) levamisole; (I) risperidone; and (J) lovastatin.

Fig. 2. Metabolic reactions of phytochemicals by gut microbiota: (A) daidzein; and (B) flavonoids: (a) flavonol; (b) flavone; (c) flavanol; and (d) isoflavone.

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Table 1. Effects of antibiotics on the gut microbiota-mediated metabolisms of drugs and phytochemicals

Drug	Reaction	Metabolite	Mode	Antibiotics (+ synergistic; -, antagonistic)	Reference
Prontosil	Azo reduction	Sulfanilamide	Bioactive	-	Fouts et al., 1957 ; Gingell et al., 1971 Gingell and Bridges, 1973
Neoprontosil	Azo reduction	Sulfanilamide	Bioactive	-	Fouts et al., 1957 ; Gingell et al., 1971
Sulfasalazine	Azo reduction	5-aminosalicylic acid	Bioactive	-	Peppercorn and Goldman, 1976; Hayllar and Bjarnason,1991; Klotz, 1985
Balsalazide	Azo reduction	5-aminosalicylic acid	Bioactive	-	Chan et al., 1983
Nitrozepam	Nitro reduction	7-aminonitrozepam	Bioactive	-	Rafii et al., 1997; Takeno et al., 1993; Takeno and Sakai, 1990
Clonazepam	Nitro reduction	7-aminoclonazepam	Toxic	-	Elmer and Remmel, 1984
Misonidazole	Nitro reduction	1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol	Toxic	-	Koch et al., 1980 ; Sheldon et al., 1984
Sulfinpyrazone	Sulfoxide reduction	Sulfinpyrazone sulfide	Bioinactive	+	Strong et al., 1987
Sulindac	Sulfoxide reduction	Sulindac sulfide	Bioinactive	+	Strong et al., 1987
Ranitidine	N-oxide reduction	Hydroxyiminoranitidine	Bioinactive	+	Basit and Lacey, 200 2 ; Machavaram et al., 2006
nizatidine	N-oxide reduction	Hydroxyiminonizatidine	Bioinactive	+	Basit and Lacey, 2001; Basit et al., 2004 ; Machavaram et al., 2006
Loperamide oxide	N-oxide reduction	Loperamide	Bioinactive	+	Lavrijsen et al., 1995 ; Kamali and Huang, 1996
Digoxin	C=C reduction and Deglycosylation	Dihydroxydigoxin Dihydroxydigoxigenin	Bioinactive	+	Lindenbaum et al. 1981 ; Magnusson et al., 1982
Zonisamie	O-N reduction/ring fission	2-sulfamoyacetylphenol	Bioinactive	+	Kitamura et al., 1997
metronidazole	C-N reduction/ring fission	N-(2-hydroxyethyl)-oxamic acid, Acetamide	Bioinactive	+	Koch et al., 1979; Kochand Goldman, 1979; Pierce et al., 2014
Lactulose	Deglycosylation	Fructose, galactose, organic acids	Bioactive	-	Elkington et al., 1969; van Berlo et al., 1988
Glucuronate-conjaged drugs: SN-38G	Deglycosylation	SN-38G→SN-38 Acetaminophen-G→ acetaminophen	Bioactive/ Toxic	-	Simon and Gorbach, 1984; Orme and Back, 1990; Adlercreutz and Martin,1980; Kehrer et al, 2001
Sodium picosulfate	Desulfation	4,4'-(pyridin-2-ylmethanediyl)-diphenol	Bioactive	-	Kim and Kobashi, 1986; Kim et al., 1992; Kobashi et al., 1986
l-dopa	Dehydroxylation	Tyramine, m-hydroxyphenylacetic acid	Bioinactive	+	Goldin et al., 1973; Peppercorn and Goldman, 1976
Flucytosine	Deamination	5-fluorouracil	Bioactive	-	Vermes et al. 2003; Harris et al., 1986
Levamizole	Ring fission and reduction	Levametabol I, II, III	Bioinactive	+	Shu et al., 1991
Risperidone	Ring fission	Dihydroxyrisperidone, Hydroxyl-keto-risperidone	Bioinactive	+	Meuldermans et al., 1994

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Lovastatin	Hydroxylation and ring fission	2-hydroxylovastatic acid	Bioactive	-	Yoo et al., 2014;
Simvastatin	Hydroxylation and ring fission	Simvastatic acid	Bioactive	-	Methaneethorn et al., 2014; Katola et al., 1998
Isoflavones	Reduction	Equol, phenolic acids	Bioactive/ bioinactive	-	Setchell and Clerici, 2010; Sepehr et al., 2009; Yokoyama and Suzuki, 2008; Kim et al., 1998
Sennosides	Reduction and deglycosylation	Sennidins	Bioactive	-	Hattori et al., 1982; Kobashi et al., 1980; Yang et al., 1996
Glycyrrhizin	Deglycosylation	18β-D-glycyrrhetinic acid	Bioactive	-	Hattori et al., 1983; Takeda et al., 1996; Kim et al., 2000
Ginsenoside Rb1	Deglycosylation	3β-D-glucopyranosyl-20S-protopanaxatriol	Bioactive	-	Akao et al., 1998; Xu et al., 2014; Joh et al., 2011
Puerarin/Daidzin	Deglycosylation, Hydroxylation, and methylation	Daidzein, calycosin, Equol, Phenolic acids	Bioactive	-	Setchell and Clerici, 2010; Sepehr et al., 2009; Yokoyama and Suzuki, 2008; Kim et al., 1998; Yasuda and Ohsawa, 1998;
Hesperidin/naringin/rutin/poncirin	Deglycosylation	Hesperetin/naringenin/ Quercetin/ponciretin	Bioactive	-	Kim et al., 1998; Jin et al., 2010
Flavonoids	Ring fission	Phenolic acids	Bioinactive/ bioactive	-	Kim et al., 1998; Selma et al., 2009; Cardona et al., 2013

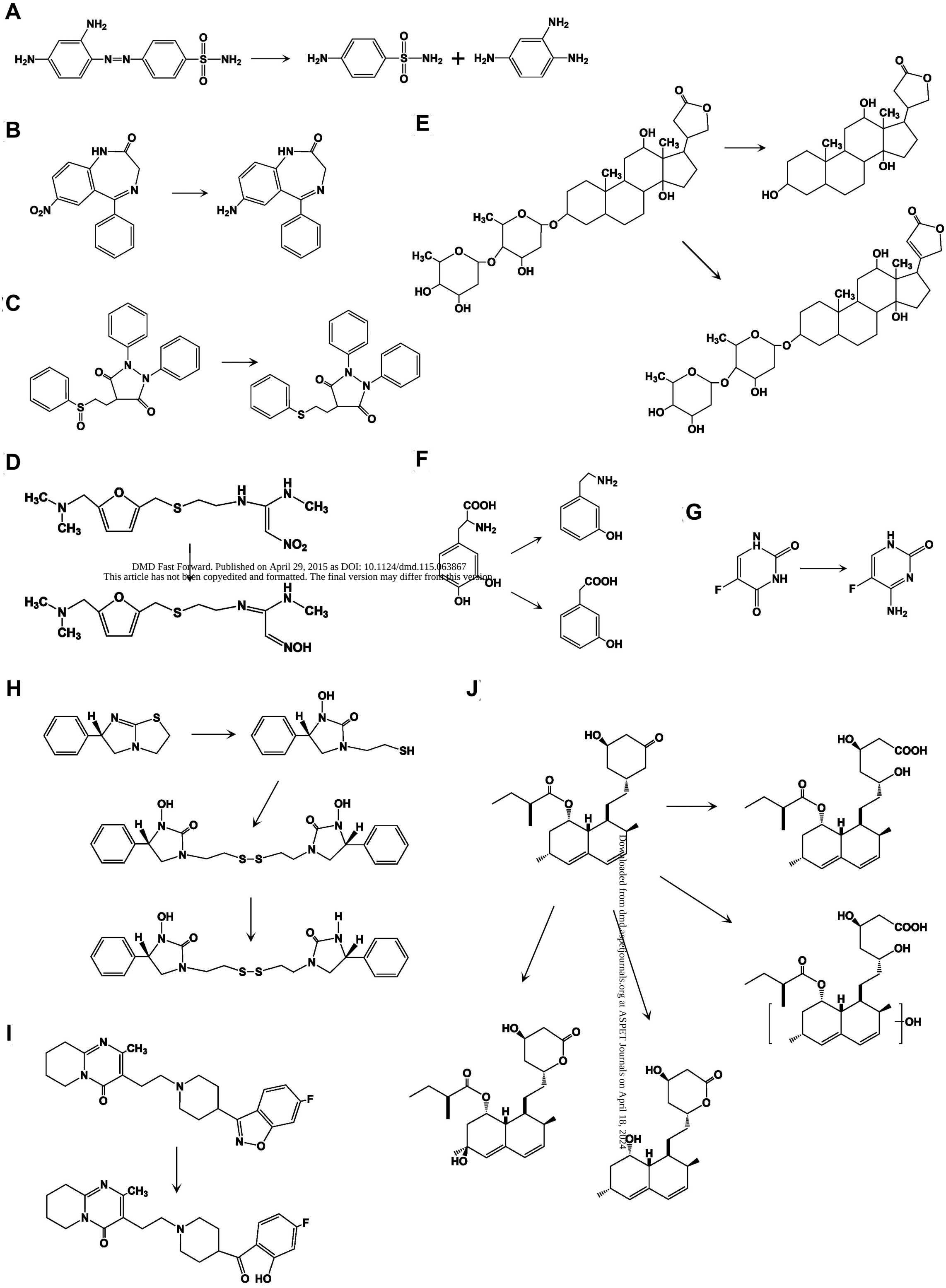

Fig. 1

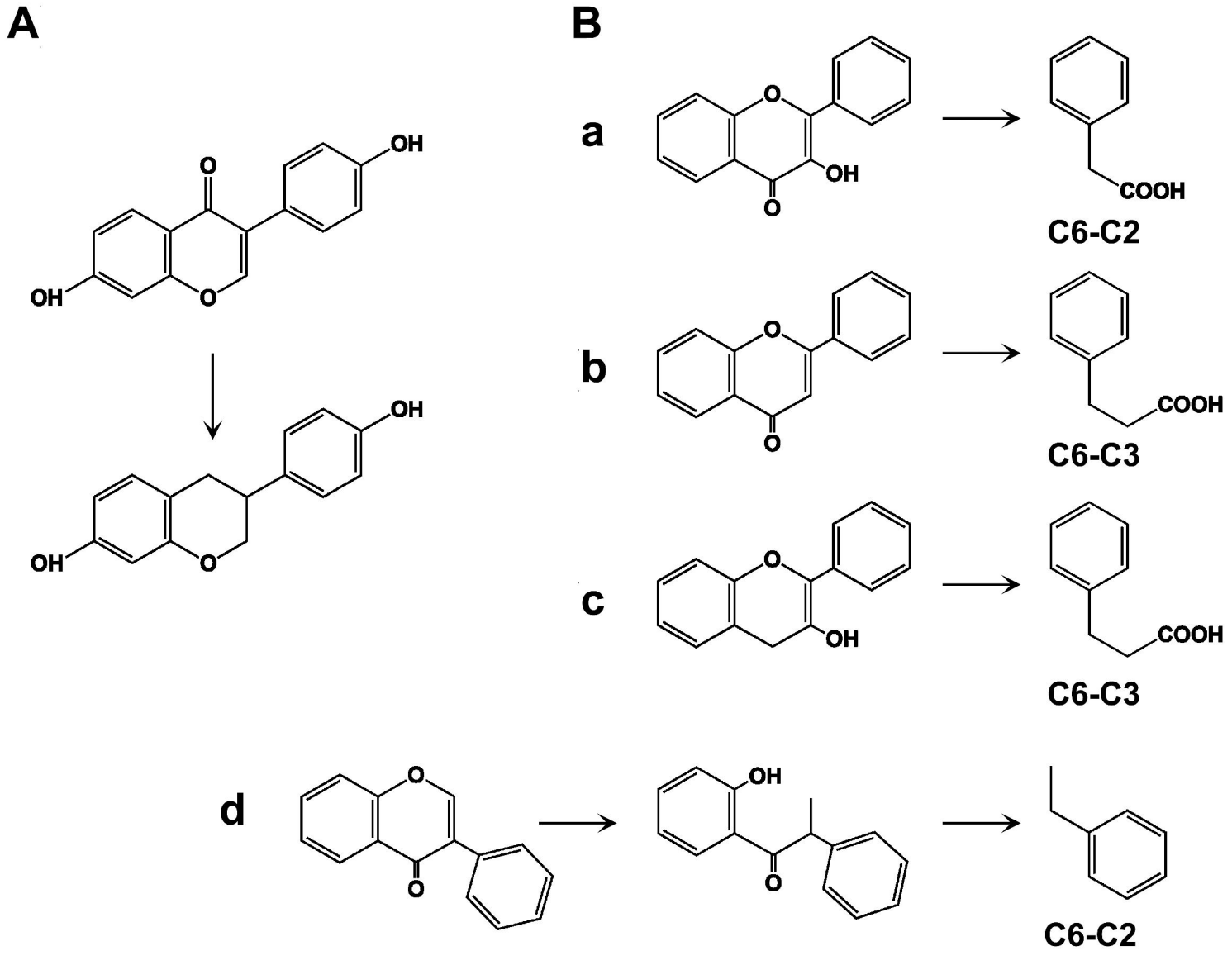

Fig. 2