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Invited Review

The role of the gastrointestinal microflora in the metabolism of drugs

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Summary

The metabolism of drugs by intestinal microorganisms is reviewed with emphasis on composition of the flora, access of drugs to their metabolic effects and methods used to study their role in drug metabolism. Types of metabolic reactions are summarized and examples of drugs subject to microbial metabolism are discussed, highlighting the influence of the drug delivery system. Pharmacological and clinical implications of metabolism of drugs by intestinal microflora are emphasized.

Introduction

It has been estimated that the human body is made up of over 10^{14} cells of which only around 10% are mammalian. The remainder are mainly the microorganisms which comprise the intestinal microflora of the host. Although these microorganisms are distributed throughout the GIT, most are found in the large intestine where they mediate hydrolytic digestive functions using carbohydrates and proteins as substrates (Cummings et al., 1989). In addition, these microorganisms have a potential to metabolize drugs and other foreign compounds that has been equated with that of the liver (Scheline, 1973).

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There are, however, some important differences. Only drugs reaching the lower part of the gut are exposed to the metabolic effect of the microflora. This is in contrast to the situation in the liver which can metabolize all drugs gaining access to the circulation. Furthermore, the majority of liver metabolic reactions involve oxidation and conjugation, whereas the microflora tend mainly to catalyze reductive and hydrolytic reactions.

Thus, the intestinal microflora represent a distinct and a potentially important site for metabolic transformation of drugs. This is particularly true when these drugs are administered in colon-specific or oral sustained release delivery systems. Therefore, consideration of the metabolic activity of intestinal microflora is highly relevant to drug delivery. Although this activity may have important pharmacological or toxicological consequences, the clinical implication of drug metab-

olism by the flora has not been thoroughly investigated in man. This is due in part to difficulties encountered in providing unequivocal evidence of a metabolic role for the microflora in humans. Recent reviews have stressed the relationship between intestinal microflora and metabolic reactions leading to the formation of toxic agents (Goldin, 1986, 1990). This review, however, considers the metabolic potential of intestinal microflora in relation to drug disposition.

Gastrointestinal Microflora

The microbial flora of the GIT constitute a very complex ecosystem containing more than 400 bacterial species (Finegold et al., 1983). Notwithstanding the complex nature of the flora, intestinal motility and the sensitivity of intestinal microorganisms to gastric acid and oxygen are the main determinants of the sites of colonization (Gorbach, 1971; Drasar and Barrow, 1985). Consequently, the nature and distribution of microorganisms vary considerably along the length of the GIT as illustrated in Fig. 1. Generally, the stomach, duodenum, jejunum and proximal ileum are sparsely populated. The flora in these parts are relatively simple and consist mainly of gram-positive aerobic or facultative species including Streptococci, Lactobacilli, and Staphylococci. The bacterial density is less than 10^4/ml of luminal content. Further along, in the distal and terminal ileum, bacterial counts are much higher (10^5–10^7/ml) and the flora closely resemble colonic flora with higher counts of coliforms and anaerobes (Finegold et al., 1983).

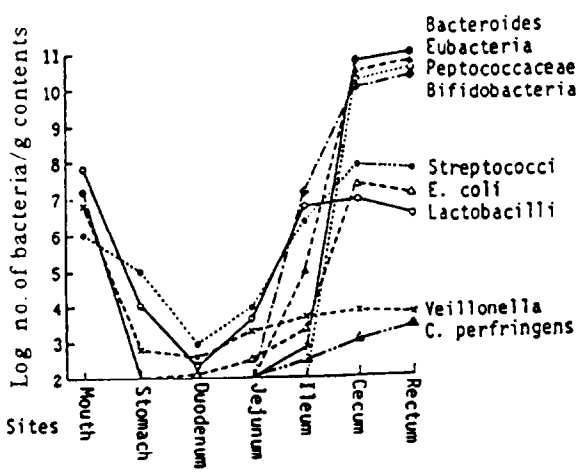


Fig. 1. Bacterial flora of various sites of intestinal tract (from Mitsuoka, 1974).

The bacterial concentration in the colon is 10^{11}–10^{12}/ml making this region the most heavily colonized part of the GIT. Although most of the different bacterial families and genera of the gut flora are found in this region, some 30–40 species make up 99% of the bacterial mass (Drasar and Barrow, 1985). The few studies that have been made on the colon indicate that it contains somewhere in the region of 220 g of wet contents (Cummings et al., 1980) of which the microbial mass is a major component forming 60% of the dry weight of colonic solids (Banwell et al., 1981). The numerically predominant species are non-spore forming anaerobes belonging to the genera *Bacteroides, Bifidobacterium, Eubacterium* and *Propionobacterium.* These species outnumber aerobes and facultative anaerobes by a factor of 10^3–10^4 (Gorbach, 1971). Anaerobic gram-positive cocci (peptococci and peptostreptococci), clostridia, enterococci and various species of enterobacteriaceae are also present (Finegold et al., 1983).

The metabolic effects of the intestinal microorganisms result mainly from the activities of the colonic microflora.

Access of Drugs to Intestinal Microorganisms

Because intestinal microorganisms are mainly restricted to the terminal part of the gut, only drugs reaching this area are susceptible to microbial metabolism. Therefore, drugs that are administered rectally and those which are not absorbed or incompletely absorbed following oral administration may be subject to biotransformation by the colonic microflora.

Incomplete absorption can arise from dissolution or permeability-rate limitations or from saturation of a carrier-mediated transport process. It can also be caused by a variety of gastrointestinal diseases.

Drugs which would normally be readily and completely absorbed from the upper parts of the GIT may come into contact with the colonic flora if they are administered in oral dosage forms intended for colon-specific (Friend, 1991) or sustained release delivery. The extent of drug exposure in this latter case is closely related to transit time of the dosage form from mouth to colon (which can be as little as 3 h; Davis et al., 1986), its colonic residence time and the rate of drug release.

Bacterial metabolism within the lumen of the GIT would seem less likely to occur for drugs which are fully absorbed from the upper parts of the GIT or are given intravenously. However, such drugs may still be exposed to metabolism by the colonic microflora after diffusion or secretion from the systemic circulation into the lumen of the GIT. In addition such drugs may also be excreted in the bile possibly as conjugates which the gut flora can metabolize to regenerate the parent compounds. Indeed, the microbial breakdown of hepatic conjugates within the bowel is an essential part of an enterohepatic circulation.

Two types of drug access to microbial metabolism can therefore be distinguished: (a) direct or presystemic access before oral absorption and, (b) indirect access resulting from drug diffusion, secretion or biliary excretion into the gut lumen. Thus, a drug showing complete oral bioavailability may still undergo substantial metabolism by intestinal microflora. This can also occur after systemic delivery by any route of drug administration.

It might be expected that microbial drug metabolism resulting from direct contact would be greater than that from indirect exposure. However, the literature contains data to support metabolism associated with both types of exposure and some examples are highlighted in this review. For some drugs exposure to GIT microflora may occur both directly and indirectly.

Methods for Studying Microbial Drug Metabolism

The techniques employed for studying the role of gut flora in drug metabolism have been reviewed by Illing (1981) and more recently by Coates et al. (1988). In general, the susceptibility of a drug to metabolism by the intestinal microflora can be investigated in vitro by incubating the drug with gut contents of an animal or man in a suitable medium. The outcome of this direct approach is usually dependent upon successful cultivation of the anaerobic microflora under conditions that approximate to the gut environment with regard to pH and redox potential. The similarities in composition of the intestinal flora of common laboratory animals and man (Drasar, 1988) suggest that data obtained from animal studies can serve as a useful background for investigations involving humans.

However, there is no assurance that a bacterial reaction demonstrated in vitro will occur in animals or man simply because the bacteria inhabit the GIT. Therefore, results of in vitro investigations must be corroborated with results from in vivo studies before assigning a role for the gut flora in drug metabolism.

Evidence for metabolism by the intestinal microflora in vivo usually relies on the detection of a specific microbial drug metabolite. The role of microflora in drug disposition is also indicated by: (a) route dependent (i.v. and oral) differences in the pattern of drug metabolism; (b) suppression of metabolite formation by administration of oral antibiotics; (c) differences in metabolite formation between colectomized subjects and subjects with intact colons; (d) variation in pattern of drug metabolism with gastrointestinal absorption site; and (e) differences in pattern of metabolism between conventional and germfree animals.

There is, however, the possibility that a drug can undergo the same metabolic transformations by both mammalian tissues and the intestinal bacteria thus limiting the utility of the above approaches. An example of such a drug is isosorbide dinitrate (Abu Shamat, 1984).

Metabolic Reactions of Intestinal Flora

The use of various methods for studying drug metabolism by the intestinal microflora as listed above has permitted the recognition of a variety

of drug metabolic reactions that can be carried out by intestinal microflora. The different metabolic transformations have been extensively reviewed by Scheline (1980). Predictably, reduction is a major reaction of the gut bacteria reflecting the anaerobic nature of the majority (> 99%) of microorganisms in the intestine. However, hydrolytic reactions and those involving the removal of various functional groups feature prominently. Examples of the main reactions and drug substrates are given in Table 1.

In some cases a drug can undergo metabolic transformations by more than one microbial reaction. For instance the metabolism of misonidazole by the microflora involves both nitro reduction and heterocyclic ring fission (Koch et al., 1980).

Factors Affecting Microfloral Metabolism of Drugs

The contribution of the intestinal flora to the metabolism of a drug depends not only upon access of the drug to intestinal microorganisms and its suitability as a substrate to microbial enzymes but also a variety of other factors.

Some of these factors are related to the human host. For instance, it has been shown that the metabolism of digoxin by the microflora exhibits intersubject (Peters et al., 1978) and interethnic variations (Alam et al., 1988) and evolves gradually during the first decade of life up to the levels seen in adults (Linday et al., 1987). The specific causes of these variations are not known.

Other factors are related to the microflora. For example the gut flora may acquire the ability to biotransform a substance following the chronic administration of that substance as a result of the emergence of new pathways of metabolism. An example of this metabolic adaptation is the formation of the suspected bladder carcinogen cyclohexylamine upon chronic administration of the artificial sweetner cyclamate (Drasar et al., 1972). The carcinogenic potential of this metabolite resulted in the banning of cyclamate in 1969.

Microbial metabolism of a susceptible drug is also influenced by both route of administration

TABLE 1

Some metabolic reactions of the intestinal microflora

Reactions	Example	Reference
Reductions		
Nitro compounds	clonazepam	Elmer and Remmel (1984)
Sulphoxides	sulindac	Strong et al. (1987)
21-Hydroxycorticoids	aldosterone	Miyamori et al. (1988)
Double bonds	digoxin	Reuning et al. (1985)
Azo compounds	prontosil	Gingell et al. (1971)
Hydrolysis		
Nitrate esters	glyceryl trinitrate	Abu Shamat and Beckett (1983)
Sulphate esters	sodium picosulphate	Jauch et al. (1975)
Succinate esters	carbenoxolone	Iveson et al. (1971)
Amides	methotrexate	Valerino et al. (1972)
Glucuronides	morphine glucuronide	Walsh and Levine (1975)
Glucosides	sennosides	Kobashi et al. (1980)
Removal of functional groups		
N-Dealkylation	methamphetamine	Caldwell and Hawksworth (1973)
Deamination	flucytosine	Harris et al. (1986)
Other reactions		
Heterocyclic ring fission	levamisole	Shu et al. (1991)
Side-chain cleavage	steroids	Cerone-McLernon et al. (1981)

and drug delivery systems. For instance Thijssen et al. (1984) showed that the oral anticoagulant acenocoumarol (nicoumalone) undergoes microbial metabolism when it is administered as crystalline powder in capsules but not when given in solution. This supports the notion that microbial metabolism is more important for slowly absorbed than for rapidly absorbed oral drug products.

The various other factors affecting the metabolic activities of intestinal microflora which include diet, drugs and gastrointestinal diseases have recently been reviewed (Mallett and Rowland, 1988; Rowland, 1988) and will not be discussed further.

Metabolism of Drugs by Intestinal Flora

In the following sections some representative drugs for which there is evidence of a role for intestinal microflora in their metabolism are reviewed. These drugs are considered from the perspective of the chemical nature and potential therapeutic implications of the microbial reactions described. The relevant data come from both in vivo and in vitro studies.

Sulphasalazine and analogues

Sulphasalazine which consists of sulphapyridine and 5-aminosalicylic acid (5-ASA) linked by an azo bond, has been used in the acute treatment and successful management of inflammatory bowel disease for well over 40 years. The drug is primarily metabolized by reduction of the azo bond to give its constituent moieties. This reaction is carried out by intestinal bacteria as established by investigations in conventional and germfree rats (Peppercorn and Goldman, 1972; Schröder and Gustafsson, 1973). This was also confirmed by anaerobic incubation studies with cultures of bacteria isolated from the gut of experimental animals and man (Peppercorn and Goldman, 1972; Azad Khan et al., 1983a). The bacterial azoreductase system catalyzing the reaction is located within the bacterial cell and requires anaerobic conditions for maximum activity (Azad Khan et al., 1983a).

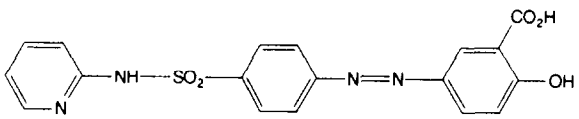
Indirect evidence supporting a role for gut flora in the metabolism of the drug in humans comes from several pharmacokinetics studies. Subjects with an intact colon excreted 61% of the dose in the urine in the form of sulphapyridine and its metabolites, whereas colectomized patients excreted only 7% of the dose in this form (Schröder et al., 1973). In patients with permanent ileostomy 75–90% of sulphasalazine was excreted unchanged in ileostomy effluents (Das et al., 1979) in contrast with normal subjects in whom only traces (less than 1%) were recovered in faeces (Schröder and Campbell, 1972). Other studies showed that the metabolism of sulphasalazine is substantially reduced in patients taking antibiotics (Houston et al., 1982) or in patients having rapid intestinal transit brought about by diarrhoea or administration of laxatives (Van Hees et al., 1979). Additionally, after oral administration of the drug its sulphapyridine metabolite appears in the systemic circulation after a lag time of 3–6 h (Azad Khan et al., 1982). This delay is caused by the passage of sulphasalazine to its site of cleavage in the colon. Collectively, such circumstantial evidence points to a vital role for the colonic microflora in the metabolism of sulphasalazine. Access of the drug to the microflora after oral administration is facilitated primarily by its poor absorption from the small intestine with 70–75% of the dose passing on to the colon and to a much lesser extent by the biliary excretion of some of the absorbed drug (Azad Khan et al., 1982).

The two metabolites of sulphasalazine which form as the result of azo bond reduction by the intestinal bacteria in the lower bowel have different fates. Sulphapyridine is almost completely absorbed and metabolized in the liver to form *N*-acetylated, hydroxylated and glucuronide derivatives which are subsequently excreted in urine along with the unchanged drug (Azad Khan et al., 1982, 1983b). On the other hand, most of the 5-ASA formed is excreted unchanged in the faeces (Peppercorn and Goldman, 1973), and about 25% is excreted in the urine following absorption and partial acetylation in the gut wall (Schröder and Campbell, 1972; Peppercorn and Goldman, 1973; Ircland et al., 1990).

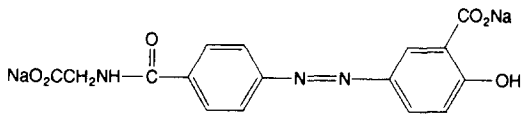
The critical role of metabolic biotransformation by intestinal microflora is indicated by clinical studies of the action of sulphasalazine in inflammatory bowel disease. These studies have concluded that this drug exerts its therapeutic effect by serving as a vehicle for the selective delivery of the active moiety 5-ASA to the colon where it can exert its local effect (Azad Khan et al., 1977; Klotz et al., 1980; Van Hees et al., 1980). This has opened up the possibility of using 5-ASA as an alternative to sulphasalazine in the treatment of Crohn's disease and ulcerative colitis. The other metabolite sulphapyridine, which appears to serve merely as an intestinal carrier for the active moiety, seems to cause most of the observed adverse effects associated with the sulphasalazine treatment (Taffet and Das, 1983).

5-ASA is rapidly and completely absorbed from the jejunum with much less absorption occurring in the ileum or colon (Nielsen and Bondesen, 1983). Since many patients prefer oral administration to topical use of enemas or suppositories, the challenge of delivery of this drug to the colon has been met with a variety of products. Enteric coated and slow release oral formulations containing 5-ASA have been developed and successfully used (Rasmussen et al., 1982; Myers et al., 1987; Rachmilewitz, 1989). These formulations offer the advantage of making the release of 5-ASA independent of the microflora and thus can deliver this substance to areas of the intestine where the flora is sparse.

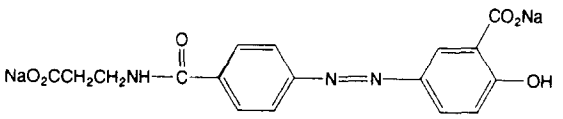
Targeting of 5-ASA to its site of action in the colon has also led to the development of a number of sulphasalazine analogues by coupling 5-ASA by an azo link to carriers less toxic than sulphapyridine. Thus, Willoughby et al. (1982) replaced sulphapyridine by 5-ASA itself to give olsalazine whereas Chan et al. (1983) used 'inert' carriers as in balsalazide or ipsalazide (Fig. 2). These prodrugs of 5-ASA are not absorbed in the small intestine but reach the colon where the microflora split the azo links to liberate 5-ASA. Faecal recovery of 5-ASA from these prodrugs has been found to be similar to that of equal doses of sulphasalazine (Chan et al., 1983; Van Hogezand et al., 1985). Effective splitting of these prodrugs is dependent on an intact colon and a



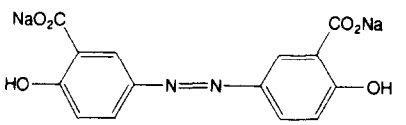
Sulphasalazine



Ipsalazide



Balsalazide



Olsalazine

Fig. 2. Structures of sulphasalazine and a selection of its analogues.

normal microflora. Unlike ipsalazide, the carrier liberated after splitting balsalazide is not absorbed to any marked extent and this analogue is currently undergoing clinical trials. However, it is unlikely that it will offer any advantage over olsalazine which provides two molecules of the active moiety for every molecule of the administered prodrug.

A recent study, the first to compare the efficacy in maintenance treatment of two of the newer 5-ASA based products, has shown that olsalazine is more effective than an enteric coated 5-ASA formulation in reducing the rate of relapse in ulcerative colitis (Courtney et al., 1992). Moreover, this prodrug appears to be more efficient in delivering 5-ASA to the colon and is associated with lower systemic absorption of the therapeutic moiety than formulations containing

5-ASA (Laursen et al., 1990). However, it is not yet clear whether the different kinetic profiles of the various 5-ASA based drugs are clinically relevant.

Digoxin

The cardiac glycoside digoxin is the most commonly used drug for the treatment of congestive heart failure. The intestinal absorption of the drug is essentially a passive diffusion process, although a saturable carrier-mediated mechanism also plays an important role (Lauterbach, 1981). Excretion is predominantly by a renal route.

Substantial metabolism of digoxin to cardio-inactive reduced metabolites (digoxin reduction products, or DRP) occurs in about 10% of patients in whom these metabolites account for 30–40% of total urinary excretion products (Peters et al., 1978; Lindenbaum et al., 1981a). The metabolism of digoxin to DRP in these patients has been shown to be solely due to metabolic activity of anaerobic gut flora (Lindenbaum et al., 1981b). The extent of formation of metabolites, however, varies with the formulation administered. Thus, consistent with their production by the microflora of the lower GIT, greater amounts of DRP form when poorly absorbed formulations are administered (Lindenbaum et al., 1981b; Rund et al., 1983). This reflects the importance of direct drug contact with the flora for the formation of DRP. However, it has been shown that the formation of these metabolites can also occur after i.v. administration of digoxin albeit to a smaller extent than after oral administration (Lindenbaum et al., 1981b). This suggests that the drug can also gain access to the microflora indirectly, possibly via secretion which seems to be an important elimination route for the drug (Lauterbach, 1981).

Eubacterium lentum, a gram-positive obligate anaerobe and a normal member of the human colonic flora, was identified as a bacterial species which can perform this metabolic conversion primarily by reducing the unsaturated lactone ring of digoxin (Dobkin et al., 1983). However, neither the presence of these organisms alone nor their concentration within the gut flora is sufficient to guarantee microbial metabolism of digoxin in man (Dobkin et al., 1983). Additional factors such as interaction between microorganisms seem to be important for the metabolic process in DRP excretors.

There is evidence to suggest, however, that extensive metabolism of digoxin by intestinal microflora can have important clinical implications. Substantial DRP formation may account for the clinical digoxin resistance and increased drug requirments in some patients (Peters et al., 1978). Lindenbaum et al. (1981a) have shown that serum digoxin levels may increase by as much as 100% when DRP formation is eliminated by commonly prescribed antibiotics. This may have serious clinical consequences in view of the low theraputic index of the drug.

Levodopa

The natural amino acid levodopa is one of the most effective agents in the treatment of Parkinson's disease. However, many factors combine to render its pharmacokinetics extremely complex. Like other neutral amino acids, absorption following oral administration appears to take place in the proximal small intestine (Rivera-Calimlim, 1972). Therefore, the rate of gastric emptying is a key factor in the absorption of the drug (Nutt et al., 1984). It is also found that the intestinal uptake of L-dopa has the characteristics of an active saturable transport process (Wade et al., 1973). Thus, competition with other neutral amino acids for intestinal transport occurs. In addition, the drug is not absorbed from the colon or rectum (Eisler, 1981) and its oral administration is associated with an extensive presystemic elimination by gut wall decarboxylase (Andersson et al., 1975).

L-dopa is metabolized by a series of reactions which include dehydroxylation to give *m*-hydroxylated metabolites. The role of the intestinal flora in these reactions was suggested by the observation that the urinary excretion of *m*-hydroxyphenylacetic acid (m-HPAA) and *m*-tyramine in Parkinsonian patients receiving L-dopa is diminished when neomycin is administered concomitantly (Sandler et al., 1971). Additionally, these metabolites are detected in the urine of conventional rats but not germ-free rats fed levodopa

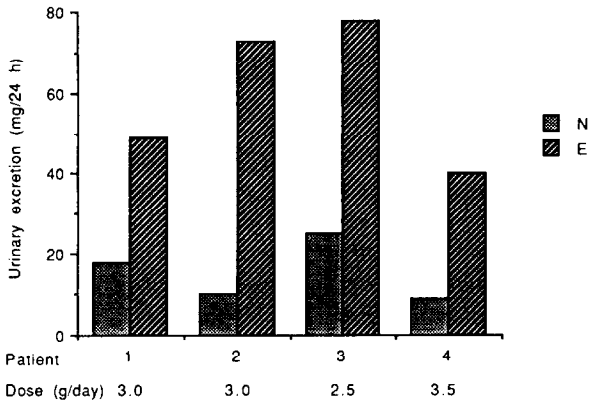


Fig. 3. Urinary excretion of m-HPAA in four Parkinsonian patients after administration of levodopa in conventional (N) and delayed release (E) tablets (adapted from data of Sandler et al., 1974).

and their formation can also be demonstrated in vitro by anaerobic incubation with caecal contents (Goldin et al., 1973). The effect of the flora in the formation of these metabolites is particularly noticeable when the drug is administered in different oral formulations. For instance, substitution of a standard tablet preparation of the drug with an enteric coated formulation in patients resulted in a substantial increase in the urinary excretion of m-HPAA (Sandler et al., 1974, Fig. 3). These findings point to a role for the intestinal flora in the metabolism of L-dopa, especially after administration of delayed release formulations.

Isosorbide dinitrate

Isosorbide dinitrate (ISDN) is among the most commonly used organic nitrates for the treatment of angina pectoris. The drug is completely biotransformed following oral administration (Down et al., 1974). Although its absolute systemic availability is 22% (Platzer et al., 1982), the systemic clearance has been shown to be greater than hepatic blood flow indicating the presence of extrahepatic sites for its elimination (Taylor et al., 1982). Many of these sites have been identified. These include the blood (Bennett et al., 1983) and intestinal mucosa (Posadas del Rio et al., 1988). The drug in addition can be metabolized in vitro by an aerobic intestinal microorganisms of both rat and man (Abu Shamat, 1984). The metabolic pathway involves step-wise denitration to produce the isomeric mononitrates and isosorbide. Since these are similar to metabolites of ISDN produced by mammalian tissues this similarity limits the utility of in vivo approaches in studying microbial metabolism of ISDN as these rely on the detection of a specific microbial metabolite of the drug.

However, circumstantial evidence of microbial involvement in metabolism of ISDN in humans comes from bioavailability studies with oral sustained release formulations of the drug. Abu Shamat (1984) showed that the bioavailability of ISDN was inversely related to extent of drug release in the lower part of the GIT despite the ability of the drug to be absorbed from the colon. Optimum bioavailability was obtained with formulations limiting drug release to 8 h. This was shown to correspond to a mean transit time of depot formulations from mouth to colon as demonstrated by using X-ray radiography. These results suggested a role for the flora in the presystemic elimination of ISDN from some formulations retarding drug release beyond 8 h. The results also indicated that such formulation cannot be used to increase dosing interval to one dose per 24 h.

Plasma concentrations ratios of the isomeric mononitrates vary with the gastrointestinal absorption site of the drug. It was suggested that a ratio of 20 and above for the 5 to the 2 isomeric mononitrates may be used as a marker indicating a contribution by the microflora in the metabolism of ISDN (Abu Shamat, 1984).

Metronidazole

Metronidazole is a 5-nitroimidazole derivative with activity against protozoa and an aerobic bacteria. It undergoes biotransformation in the liver by side-chain oxidation and glucuronide formation. The unchanged drug and its nitro-containing oxidative metabolites are excreted in the urine (Stambaugh et al., 1968). The drug can in addition undergo reductive metabolism. Small amounts of reduced metabolites, acetamide and *N*-(2-hydroxyethyl)oxamic acid, have been found in the urine of patients taking metronidazole

(Koch et al., 1981). These reduced, metabolites appear in the excreta of conventional rats but not of germ-free rats and are also formed by anaerobically incubating metronidazole with pure and mixed cultures of gut flora (Koch and Goldman, 1979; Koch et al., 1979). These results indicate an obligatory role for the microflora in the formation of these metabolites. Access of the drug to the flora is probably facilitated by its secretion from the systemic circulation into the gut lumen (Ings et al., 1975).

In a recent study correlating the systemic availability of metronidazole with the retardation of drug release the reduced bioavailability of the drug from some oral sustained release formulations has been attributed to the metabolic activity of the intestinal microflora (Shamat, 1992).

Sulphinpyrazone

Sulphinpyrazone is a uricosuric agent but in recent years has also been used in treatment of thromboembolic disorders because of its potential to inhibit platelet aggregation (Anturan Reinfarction Italian Study Group, 1982). This activity is probably due to its sulphide metabolite which has a longer half-life than the parent sulphoxide compound and has been reported to be up to 16-times more potent than the drug at inhibiting platelet aggregation (Del Maschio et al., 1984).

Studies in the rat (Renwick et al., 1982), rabbit (Strong et al., 1984a). and in humans (Strong et al., 1984b) have shown that intestinal microflora are exclusively responsible for reduction of the drug to the sulphide metabolite. Despite the formation of this metabolite by the intestinal flora, the amounts formed in humans have been reported to be similar after both i.v. and oral tablet administrations (Strong et al., 1984b). This suggests that the drug comes into contact with the microflora indirectly either via the bile or by simple diffusion into the gut lumen. However, increasing the delivery of sulphinpyrazone to the large intestine by administering the drug in a sustained release formulation has been shown to be associated with enhanced formation of the active metabolite as a result of the increased duration of contact with the microflora. Therefore, other factors and conditions which affect the gut flora or drug delivery to the large intestine might affect its anti platelet activity by altering the formation of the active sulphide metabolite. Thus, whilst negligible amounts are formed in patients on antibiotics or ileostomized patients, increased amounts are formed in those receiving metoclopramide which increases intestinal motility (Strong et al., 1984b, 1986).

It is interesting to note, however, that although in vivo studies have demonstrated a prominent role for anaerobes in the reduction process, in vitro tests with single strains of faecal bacteria have shown that the aerobic bacteria are predominantly responsible for this reduction (Strong et al. 1987). It was suggested that the discrepancy between in vitro and in vivo results may be due to the very large number of anaerobes present in the hind gut which produce an anaerobic environment suitable for other organisms.

Flucytosine

Flucytosine is a systemic antifungal drug which is rapidly and effectively absorbed from the GIT and about 90% of which is excreted unchanged in the urine (Cutler et al., 1978). It acts by interference with nucleic acid synthesis in fungal cells. Cytosine deaminase in susceptible fungi converts flucytosine to 5-fluorouracil (5-FU) which may then be incorporated into fungal RNA or can be further converted by a series of metabolic reactions to produce an inhibitor of DNA synthesis (Lyman and Walsh, 1992). Mammalian cells cannot convert the drug to the cytotoxic 5-FU thus providing a mechanism for selective toxicity of this drug to fungi. However, toxic adverse effects similar to those associated with 5-FU treatment can occur in patients receiving flucytosine (Kauffman and Frame, 1977). These include dose-dependent bone marrow suppression.

Diasio et al. (1978) investigated the possibility of conversion of flucytosine to 5-FU in patients and healthy subjects given flucytosine. This possibility was confirmed by the presence of 5-FU in the serum of all subjects studied often at levels comparable to those found during 5-FU chemotherapy. It was concluded that 5-FU may account for some of the toxicity associated with flucytosine. The site of conversion of flucytosine to 5-FU

in humans is not known. However, it has been reported that oral administration of flucytosine to rats results in its partial deamination to 5-FU possibly by intestinal microflora (Koechlin et al., 1966). This and the lack of cytosine deaminase activity in mammalian cells (Diasio et al., 1978) prompted Harris et al. (1986) to investigate the metabolism of the drug by human intestinal microflora using an in vitro semicontinuous culture system. It was found that microflora can be induced after chronic exposure to metabolize the drug to 5-FU. It was suggested that this microbial conversion of flucytosine to 5-FU may provide a mechanism through which the toxicity of the drug is manifested. However, the role of microflora in the metabolism of flucytosine to 5-FU needs to be established in vivo in man before assigning a more definitive role for the microflora in the toxicity of the drug.

Conclusions

Drug metabolism studies usually concentrate on transformations by mammalian enzymes. It is now evident from in vivo studies in man that the intestinal microflora can perform a large number of metabolic reaction with drugs of different chemical structures and pharmacological properties. These reactions are generally at variance with mammalian processes and can result in drug activation, inactivation or the formation of toxic metabolites. Studies investigating the possibility of microbial adaptation as a result of chronic drug administration are greatly needed.

Although the intestinal microflora are mainly restricted to the terminal part of the GIT, exposure of drugs to their metabolic effects may occur after systemic drug delivery by all routes of administration. Therefore, consideration of the metabolic reactions of gut microflora in drug development studies is necessary to assess their possible implications.

The biotransformation of drugs susceptible to microfloral metabolism is influenced by diverse factors which can affect both drug accessibility to the microflora and extent of metabolism. An appreciation of these factors may result in better assessment of their possible therapeutic effect and can assist in the development of reliable prodrugs or dosage forms that can be microbially triggered to achieve colon-specific drug delivery.

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