

Bioavailability File: Metronidazole

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Summary

Metronidazole is an antimicrobial nitroimidazole derivative, which was originally introduced to treat *Trichomonas vaginalis* but nowadays is used for the treatment of anaerobic and protozoal infections. Metronidazole is bactericidal through toxic metabolites, which cause deoxyribonucleic acid (DNA) strand breakage. It has a bioavailability of more than 90% after oral administration. The drug is metabolized by the liver and the hydroxy-metabolite has also a therapeutic effect. In this review, the physicochemical structure, assay methods, pharmacological and pharmacokinetic properties, and bioavailability of metronidazole are summarized.

Key Words: Metronidazole, bioavailability, pharmacokinetics, pharmacology, drug interactions.

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Özet

Metronidazol ilk olarak *Trichomonas vaginalis* tedavisi için çıkarılmış, ancak günümüzde anaerobik ve antiprotozoal enfeksiyonların tedavisinde kullanılan antimikrobiyal bir nitroimidazol türevidir. Metronidazol, deoksiribonükleik asit (DNA) zincirinin kırılmasına sebep olan toksik metabolitleriyle bakterisidal özellik gösterir. Oral yolla verildiğinde % 90'dan fazla biyoyarlanım göstermektedir. Karaciğerde metabolize olur ve ilacın hidroksi metaboliti de terapötik etkiye sahiptir. Bu derlemede, metronidazolün fizikokimyasal yapısı, miktar tayini yöntemleri, farmakolojik ve farmakokinetik özellikleri ile biyoyarlanımı özetlenmiştir.

Anahtar kelimeler: Metronidazol, biyoyarlanım, farmakokinetik, farmakoloji, ilaç etkileşimleri.

INTRODUCTION

Metronidazole was introduced in 1959 for the treatment of patients with *Trichomonas vaginalis* and it has since been evaluated in the treatment of infections caused by anaerobic bacteria¹. It is a member of the 5-nitroimidazole antimicrobials class, especially fatal on some protozoa². It has been used successfully in the treatment of vaginal infections, antibiotic-associated pseudomembranous colitis, trichomoniasis and symptomatic amebiasis^{3,4}. It is a drug of first choice in the infections of *Helicobacter pylori*⁵⁻⁷. It has also been reported to be of value in Crohn's disease⁸⁻¹⁰.

It is usually absorbed well (80-90%) by oral route. The principal route of elimination is hepatic oxidation and glucuronidation¹¹. Metronidazole has common adverse effects like nausea, diarrhea, anorexia, vomiting and urticaria, although it is widely used¹². The carcinogenic potential and effects on spermatogenesis are under investigation¹³.

Physicochemical Properties

Metronidazole is named as 2-methyl-5-nitroimidazole-1-ethanol or 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. Its formula is C₄H₉N₃O and the chemi-

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cal formula can be seen in Figure 1. Molecular weight is 171.2.

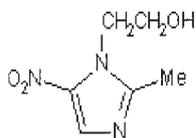


Figure 1. The chemical structure of metronidazole.

It is white to pale yellow, odorless and in crystal or crystalline powder form, melting between 159-162°C. It is sparingly soluble in water, alcohol or chloroform and slightly soluble in ether¹⁴⁻¹⁶. Its pKa was detected as 2.5¹⁷

Metronidazole was reported to undergo hydrolysis in aqueous media due to the presence of photolytically generated hydroxyl radicals. Light irradiation has more effect on the degradation of metronidazole in solution than irradiation with sonic energy. Wang et al.¹⁸ studied the degradation kinetics under various conditions such as pH, total buffer concentration, ionic strength, temperature, light exposure and cosolvent system. The results indicated pseudo-first-order degradation kinetics for metronidazole in aqueous solution. In the pH range between 3.9 and 3.6, metronidazole was more stable than in other pH regions. There was not enough degradation in a 50-day period for both light-exposed and light-protected samples. However, UV irradiation did accelerate the degradation processes of metronidazole in the light-exposed samples. The effect of gamma rays on metronidazole was investigated and it was found that gamma irradiation of metronidazole produced free radicals¹⁹. In another study, metronidazole was found to become biologically inactive when it was impregnated into solid sensitivity discs and then exposed to light. The effect of light on parenteral solutions of metronidazole caused a fall in pH, an increase in nitrite ion concentration and formation of amber discoloration²⁰.

From a thermodynamic study, it was concluded that aqueous suspensions of the anhydrous form of metronidazole benzoate were metastable and that storage of such suspension at temperatures lower than 38°C leads to hydrate formation accompanied by crystal growth²¹.

Analytical Methods

Metronidazole can be determined with microbiological techniques, spectrophotometric methods, thin layer (TLC), gas (GC) and high-pressure liquid chromatography (HPLC)²²⁻²⁵. Early detection of metronidazole was based on bioassay and gas-liquid chromatography. These methods were time consuming and failed to determine the concentrations of the metabolites. UV (ultraviolet) and IR (infrared) spectrophotometry and HPLC, especially for analysis from biological fluids, were preferred with the development of the other techniques²⁶⁻³¹. HPLC has many advantages over the other methods as an accurate and sensitive method. It was reported that HPLC method has sensitivity at ng/g level to detect drug content from vaginal tissue³² and as little as 5 ng of the drug was detectable from serum³³. HPLC methods are able to determine hydroxy and acetic acid metabolites. Detection is usually carried out at 320 nm³⁴.

Pharmacology

Metronidazole and nitroimidazoles are thought to produce their bactericidal activity through four phases.

- 1 - entry into bacterial cell
- 2 - nitro group reduction
- 3 - action of the cytotoxic by products
- 4 - production of inactive end products

The selective toxicity of nitroimidazoles depends on two factors. The first is the reduction of nitroimidazoles^{2,35,36}. Since metronidazole is a small, lipophilic molecule that cannot ionize, it can easily enter into the microorganism cell with passive diffusion. Nitroimidazole sensitive cells are commonly anaerobic and include low redox potential proteins with a role in electron transfer. The second mechanism is to turn the nitro group of nitroimidazoles into intermediary toxic metabolites with the reduction caused by non-enzymatic chemical reactivity.

This toxic metabolite interacts with DNA, RNA (ribonucleic acid) or intercellular proteins, but the ma-

major effect appears with the breakage of the DNA strand. Therefore, the inhibition of DNA synthesis causes the death of the cell. The therapeutic dose of the drug affects the lymphocytical DNA²⁻³⁷. Another mechanism is the self-reduction potential of nitroimidazoles that destroys intercellular electron transfer and depresses of NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate). As a result, the energy formation is inhibited². Both clinical and microbiological resistance has been demonstrated rarely. However, the study of Edwards et al.³⁸ has shown that microorganisms like *E. coli*, *Proteus* and *Klebsiella* absorb metronidazole in the treatment of *T. vaginalis*. Therefore, the concentration of active substance in vaginal fluid has been decreased.

The minimum effective concentration of metronidazole has been determined as 0.1-8 µg/ml, and general oral doses for 5 to 10 day treatment periods vary between 400-800 mg. Serum concentration and dose range should be adjusted for newborns and children. The adjustment has been made according to body weight (35-50 mg/kg), because of the similarity of the clearance as in adults. There is no need to change the dose during pregnancy since there is not a significant difference in pharmacokinetics. The drug can be administrated by intravenous infusion with a rate of 5 mL/min. every 8 hours (h), if the oral route is not available. Metronidazole can be administered rectally 1 g, 3 times a day for 3 days^{16,39-41}. The oral dose in acute ulcerative gingivitis is 600 mg per day. The drug can be given at a dose of 500 mg for 7 days vaginally. Solutions of 1% and gels at different ratios have also been used. It has been reported that 500 mg metronidazole can be effective topically, once or twice a day¹⁴.

Adverse Effects

The adverse effects of metronidazole are generally dose-related. The most common untoward effects are nausea, diarrhea, anorexia, epigastric distress and abdominal cramps. Urticaria, pruritus, flushing, dry mouth, dry vulva and vagina, feeling of pelvic pressure, vertigo, headache, ataxia and insomnia occur occasionally. The urine sometimes becomes dark

in color^{14,39}. These adverse effects involve the gastrointestinal tract and nervous system especially with high doses³³. Reduction of side effects while prolonging its action by using controlled release of oral dosage forms is highly desirable. Several natural and synthetic polymers like HPMC (hydroxy propyl methyl cellulose) can be used to modify the drug release⁴².

Metronidazole is mutagenic to some bacteria species. In large doses administered to rodents, metronidazole interferes with spermatogenesis and is carcinogenic¹. Metronidazole passes through the placenta and has the risk to affect the fetus. There are papers recommending that the drug not be used during pregnancy^{3,43}. In one study, at the maximum concentration of metronidazole that can dissolve in water (10 mg/ml), no toxicity was detected in rabbit and human sperm⁴⁴. It has been reported that although metronidazole itself may not be carcinogenic, its effects on DNA can accelerate carcinogenicity. Unfortunately there are no studies yet reporting patients treated with metronidazole who were followed for a sufficient period of time; the available data suggests it would take 20 years to determine if metronidazole is carcinogenic⁴⁵.

Pharmacokinetics and Bioavailability

Absorption

The absorption of metronidazole has been studied in a variety of dosage forms including oral tablets, infusions, vaginal-rectal suppositories, and topical gel. The oral absorption of metronidazole is excellent, with bioavailability often reported as >90%⁴⁶⁻⁴⁸. The peak plasma drug concentration (C_{max}) after a single dose of 500 mg is approximately 8 to 13 mg/L, with a corresponding time (t_{max}) of 0.25 to 4 h^{49,50}. The correlation between C_{max} and t_{max} can be seen from the plasma concentration-time graphic of four healthy males given a single dose of 500 mg metronidazole (Fig. 2).

The suspension of benzyl metronidazole, equivalent to 400 mg or 2 g metronidazole, was given to male volunteers. The obtained peak plasma concentration

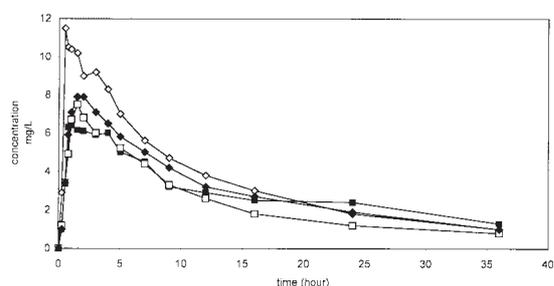


Figure 2. The plasma concentration-time graphic of four healthy volunteers who received 500 mg metronidazole as an oral single dose.

was 4.6 mg/L for 400 mg and 17 mg/L for 2 g, and t_{max} was found as 3.2 and 5.1 h, respectively. The bioavailability of this formulation was 20%, low when compared to metronidazole, and C_{max} was 45% decreased⁴⁶.

Serum concentrations of metronidazole have been evaluated in healthy female volunteers who received a single dose of 2 g metronidazole orally. C_{max} and t_{max} were found as 40 mg/L and 1-2 h, respectively⁵¹. A group of 121 women undergoing elective gynecologic surgery were evaluated for the potential of prophylactic use, after receiving oral metronidazole, intravenous metronidazole and placebo. This study revealed that the incidence of postoperative infections could be reduced with the use of metronidazole. The blood levels of metronidazole showed that the oral use of drug is as effective as intravenous use (Table 1)⁵².

Table 1. Post- and intra-operative blood concentration values of metronidazole given for prophylactic purpose by oral and iv route for three days

Administration route	Post-operative			
	Intra-operative (mg/L)	1 st day (mg/L)	2 nd day (mg/L)	3 rd day (mg/L)
IV	22.1 ± 0.9	15.2 ± 0.8	1.5 ± 0.2	0.3 ± 0.1
Oral	17.9 ± 1.3	3.4 ± 0.3	0.3 ± 0.1	0

In one previous study Flagyl suspension and test suspension were compared as fractions absorbed from various parts of the gastrointestinal system (Fig. 3). It was determined that the absorption of drug was sensitive to permeability; however, it was not affected by the transit time through small intestine⁵³.

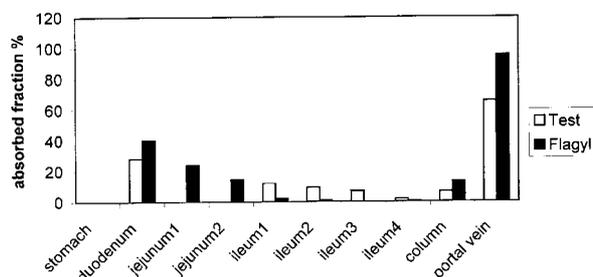


Figure 3. % Fraction of metronidazole absorbed from different parts of gastrointestinal tract.

Pharmacokinetic characteristics of metronidazole were studied in 19 pregnant women. All patients received 250 mg or 1 g metronidazole orally in a single or in multiple doses for 10 days. Serum concentrations were found as 4.6 and 17.4 mg/L, respectively⁵⁴.

Although metronidazole is generally given orally the use of vaginal and rectal suppositories is an alternative route, especially when local effect is required. Suppositories prepared with Witepsol H15, containing 500 mg metronidazole, were studied quantitatively. The serum concentration of drug was evaluated every 8 h and compared to the intravenous route. It was claimed that the suppositories were as effective as infusions of the drug⁵⁵. When 500 mg metronidazole was administered rectally, C_{max} and t_{max} reached 4-5.5 mg/L and 0.5-1h. The absorption of metronidazole released from the suppositories through the rectum was found as 67-82%⁵⁶ (Fig. 4).

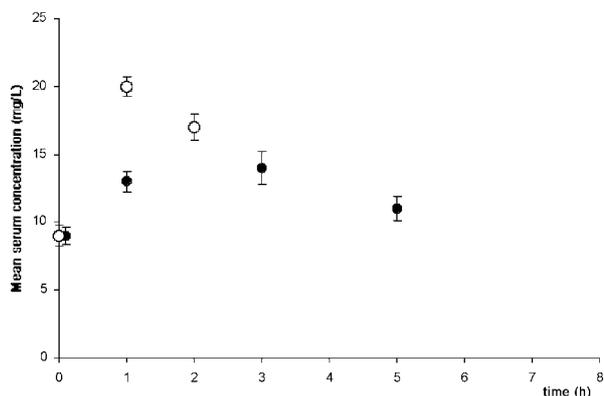


Figure 4. The serum concentration time graphic of 500 mg metronidazole after rectal () and intravenous () administration.

In one study, the vaginal suppositories of 500 mg metronidazole show a bioavailability between 20-25 %⁵⁷. In another the vaginal bioavailability was found as 56% when compared to 500 mg intravenous dose⁵⁸.

In the study of Fredericsson et al., the blood level of metronidazole administered orally and vaginally was found closer to the level obtained after intravenous injection⁵⁹. It is known that since the diffusion rate of dissolved drug from the diffusion membrane is a rate-limiting factor, intravaginal absorption is variable depending on the base used in the formulation⁶⁰. When the biodistribution of vaginal suppositories of technetium-99m labeled metronidazole prepared with Witepsol H15 was investigated with gamma camera, 25% of the drug was found in the urinary bladder 2.5 h after administration⁶¹. When 5 g gel containing 0.75% metronidazole was administered, a value of 0.2-0.3 mg/L for C_{max} and 8.3-8.5 h for t_{max} were observed. C_{max} and t_{max} values gained from vaginal suppositories and inserts containing 500 mg drug were 1.1-1.9 mg/L and 7.7-20 h, respectively^{57,62,63}. In another study it was concluded that vaginal application of 500 mg metronidazole daily for seven days is equally effective as oral administration in the treatment of bacterial vaginosis⁴⁶. The determined metabolites of the drug delivered vaginally were less when compared to iv and oral administration. However, the two metabolites show a constant ratio⁶². A slight systemic absorption was reported from the topical application of metronidazole. The serum concentration was found as 66 mg/L 24 h after the application of 1 g gel containing 0.75% metronidazole in adults⁶⁴.

Distribution

Metronidazole has generally been reported to have good penetration into the cerebrospinal fluid (CSF) and central nervous system (CNS). A patient with *Fusobacterium meningitis* had CSF concentrations of 13.9 and 11 mg/L at 2 and 8 h, respectively, after oral administration of 500 mg doses twice daily⁶⁵.

Protein binding of metronidazole is less than 20%^{50,66,67}. The reported volumes of distribution

(Vd) in various studies have ranged from 0.65 to 0.71 L/kg in newborn infants, to 0.51 to 1.1 L/kg in adults. Single dose studies with oral and intravenous 500 mg metronidazole have determined the area under the serum concentration-time curve (AUC) to be approximately 100-159 mg/L.h^{47,48,68}. Pregnant women tend to have AUCs that also fall in this range, but children and infants may have higher AUCs depending on the dose utilized^{49,69,70}. Sixteen pregnant women prepared for cesarean sections received a single intravenous dose of metronidazole 500 mg. Arterial cord blood concentrations at the time of cesarean ranged from 8.9 to 16.4 mg/L. Placental tissue concentrations have been reported as being relatively low compared with serum concentrations⁷¹. Concentrations of metronidazole as single 400 mg oral doses or 4 x 400 mg doses given every 8 h were administered to 30 women who required hysterectomies. Serum and tissue samples from the fallopian tubes and uterus were obtained 3-4 h after administration. The concentration in the uterus and fallopian tubes were 94% and 97.3%, respectively⁷². Thirty women undergoing laparoscopy received metronidazole 500 mg intravenously as part of prophylaxis regimen. The average time elapsed from infusion of metronidazole until simultaneous sampling of peritoneal fluid and blood was 55 minutes. The averaged serum concentrations were 10.7 mg/L and peritoneal fluid concentration was 7.2 mg/L⁷³. When the rate and extent of transvaginal absorption and the disposition of ¹⁴C labeled metronidazole were compared after 6 h intravaginal application to rabbits, the levels of labeled drug were highest in the urinary bladder, liver and kidney⁷⁴.

The pancreatic tissue concentration of drug in eight patients with pancreatitis and pancreatic carcinoma after 500 mg iv administration was 5.1 mg/L⁷⁵. Twelve patients with necrotizing pancreatitis had serum and necrotic tissue samples obtained to determine tissue penetration. The penetration of metronidazole was 99% with a concentration of 8.4 µg⁷⁶. Twelve patients undergoing colorectal surgery received 1 g iv metronidazole for prophylactic purpose. Concentration of colonic mucosa and abdominal wound closure were found as 0.94 and 0.76⁷⁷. Seventeen patients under going the same operation received 1.5 g iv metronidazole and the concentrations

of serum and tissue samples (colon, peritonum, ileum, muscle, omentum, appendix and subcutaneous fat) were determined for 72 h. Concentrations of metronidazole and hydroxy metabolite were assayed. The mean value of drug in the various tissues was in the 10-30 mg/g range⁷⁸. Metronidazole concentrations were estimated in four human volunteers after a single dose of 750 mg taken orally. Samples of blood, saliva and gingival fluid were collected and C_{max} for saliva and plasma were in the same range⁷⁹.

Metabolization

Metronidazole is metabolized in the liver into two major metabolites. These are (1-(2-hydroxy-ethyl)-2-hydroxy-methyl-5-nitroimidazole) hydroxy-metronidazole and 2-methyl-2-nitroimidazole-1-acetic acid. The acetic acid metabolite is only found in urine and does not possess any pharmacological activity. However, hydroxy-metronidazole has an antimicrobial potency approximately 30% that of metronidazole against certain strains of bacteria and can be detected readily in the systemic circulation^{80,81}. The acetic acid metabolite has only 5% of the activity of the parent drug and is only detectable in patients with renal dysfunction. Glucuronide and sulfate conjugates and an oxidation product have also been detected⁸². The metabolization tract and chemical formulas can be seen in Figure 5.

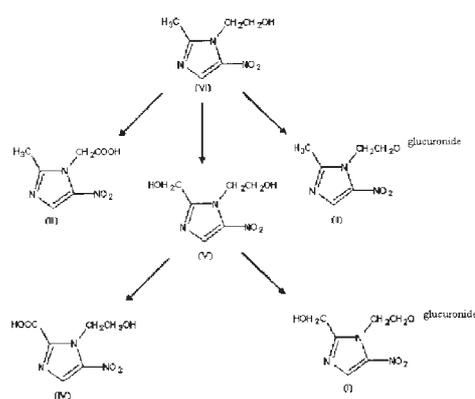


Figure 5. The metabolization pathway of metronidazole in humans and chemical structure of metabolites.

VI: metronidazole V: 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole; IV: 1-(2-hydroxyethyl)-2-carboxylic acid-5-nitroimidazole; III: 1-acetic acid-2-methyl-5-nitroimidazole; I and II are glucuronide conjugates.

Elimination

The main routes for the elimination of metronidazole are hepatic oxidation and glucuronidation. Its low molecular weight, trivial protein binding and relatively high renal clearance argue against significant biliary excretion¹². The therapeutic plasma concentration for metronidazole is approximately 5 µg/mL. An average of 71.1% of an intraduodenal or intravenous dose of ¹⁴C labeled metronidazole was excreted in 24 h, 23.9% in bile and 47.6% in urine, over 5 days⁶⁷. 5% of the drug was turned into carbon dioxide with the breakage of the imidazole ring by gut flora⁸³. 7-12% of the dose was found unchanged in urine⁸⁴. 14-24.1% of the applied dose was found as hydroxyl and 9.6-12% as acid metabolite^{46,85}. The total clearance of metronidazole from serum has been reported to range from 2.1 to 6.4 L/h/kg body weight^{86,87}. Metronidazole shows dose-dependent clearance between 250-2000 mg. The half-life of the drug ($t_{1/2\beta}$) ranges from 6-10 h. Many studies report $t_{1/2\beta}$ as 8 h in adults and 22 h in newborn^{25,88,89}. Thiercelin et al.⁹⁰ noted that metronidazole taken 500 mg orally every 8 h resulted in AUC values 51% higher than the same dosage administered intravenously, even though the $t_{1/2\beta}$ was 6 h for both routes. There are two explanations for this:

1-Metronidazole inhibits its own metabolism by gut microsomal monooxygenases.

2-Oral administration leads to excessive glucuronidation with resulting entero-hepatic circulation.

The $t_{1/2\beta}$ of the hydroxy metabolite is longer than that of metronidazole and is between 8.5-19.2h⁹⁰. A single dose (500 mg) of intravenous metronidazole was administered to six patients with $CL_{CR} \leq 0.6$ L/h because of acute renal failure, for four days. The distribution value (V_d) was 0.65 L/kg, $t_{1/2\beta}$ was 9.9 h, total plasma clearance was 55.5 ml/min. and non-renal clearance was 54.0 ml/min⁹¹. In a similar study, 29 patients with renal insufficiency were given a single intravenous dose of 500 mg metronidazole, and plasma and urinary concentrations of the drug and two major metabolites were determined.

The pharmacokinetic parameters were not significantly affected by renal failure of any degree⁹². The patients with creatinine clearance (CL_{CR}) ≤ 1.8 L/h had to be kept under control; metronidazole must not be administered to patients with $CLCR \leq 0.6$ L/h because of the accumulation of the metabolites of the drug⁹³.

The influence of hemodialysis has also been evaluated for metronidazole and its metabolites. 500 mg of the drug was administered intravenously to five volunteers with normal renal function, four patients with renal failure and five patients requiring hemodialysis. The $t_{1/2\beta}$ was 6.13-6.8 h, V_d was 1.29-1.44 L/kg, and $CLCR$ was 9.1-11.02 L/h for all groups, but metabolite concentrations increased three-fold in the group with renal failure⁹⁴. The pharmacokinetic parameters of metronidazole were determined in patients with liver disease. It was observed that liver disease did not markedly influence the disposition of single oral doses of metronidazole⁹⁵. The bioavailability was very good in Crohn's disease and ulcerative colitis. There was no difference in half-life, V_d or plasma clearance⁹⁶.

Food and Drug Interactions

Metronidazole, which does not interact with other drugs, may provoke a disulfiram-like reaction with ethanol in some individuals^{73,87}. The synergistic effect of proton pump inhibitors and antimicrobial substances has been investigated. Single doses of 400 mg metronidazole were administered intravenously to 24 healthy men while taking placebo or omeprazole. Omeprazole had no influence on the plasma kinetics of metronidazole. However, metronidazole AUC was reduced in gastric juice and C_{max} was decreased. These results indicate that low pH ionizes metronidazole and hydroxy metabolite, leading to high concentrations in gastric fluid that are reduced when gastric pH is lowered by omeprazole⁹⁷.

The possible influence of food intake on the bioavailability of metronidazole was examined in 10 healthy volunteers by administration of a single dose of drug, on an empty stomach, or with a standardi-

zed breakfast. It was considered that food intake did not significantly alter the bioavailability of metronidazole. Metronidazole can be taken with and between meals safely. Bioavailability is not under the influence of food intake but absorption can be delayed¹⁴.

Targeting Studies

Matrix tablets of metronidazole were prepared using guar gum as a carrier to develop colon targeted delivery systems. The release studies were conducted in stomach and small intestine. The minimal release of drug in the first five hours provided the local action required in the colon⁹⁸. Two types of alginate gel beads capable of floating in the gastric cavity were prepared using metronidazole as a model drug. The release properties have shown that alginate gel beads were suitable not only for sustained release of drugs, but also for targeting the gastric mucosa⁹⁹.

RESULTS

Metronidazole is the first-choice drug in the treatment of anaerobic infections because of its pharmacodynamics and pharmacokinetics, acceptable adverse effect profile and undiminished antimicrobial activity. The other advantages in using metronidazole are its availability in many dosage forms, good tissue penetration and low expense. Although newer agents have curtailed the use of metronidazole, it still has a role in treatment of various infections. The new combinations did not show any therapeutic advantage over metronidazole. Therefore, many clinicians consider metronidazole as the gold standard antibiotic with its anaerobic activity. However, to obtain an effect at the desired region without getting involved with systemic circulation is still the main goal in pharmaceutical design of the drugs. The modified release formulations appear to be a promising vehicle for delivering the drugs to the desired region and reducing the adverse effects. These modifications, like sustained release, delayed release, etc., may be beneficial for metronidazole use in infections like Crohn's disease, in which a specific drug delivery regimen should be considered.

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