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Research paper

Enhancement of oral absorption of metronidazole suspension in humans

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Abstract

The purpose of this study was to investigate oral absorption of two metronidazole suspension products, Flagyl® and a test product. Twenty-four healthy volunteers participated in the study. The crossover study was done in a two-phase, two-sequence manner with a 2-week washout period. Individual disposition kinetics were determined by non-compartmental analysis. $AUC_{0-infinity}$ and C_{max} values were 1.26 and 1.86 times more in the case of test formulation. Mean plasma drug concentrations were analyzed to estimate the rate and extent of oral absorption. The optimized duodenal, jejunal1, jejunal2, illial1, ileal2, ilial3, ilial4, colonic permeability values ($\times 10^{-4}$) for the test and Flagyl products were 3.96, 3.96, 3.96, 0.68, 0.37, 0.01, 0.12, 0.38 and 2.34, 0, 0, 1.2, 1.1, 0.9, 0.3, 0.04 cm/s, respectively. The total fraction of oral dose absorbed for the test and Flagyl products were 95.5% and 65.6% respectively, consistent with the pharmacokinetic ratios. The test product exhibited significantly higher absorption rate and extent than Flagyl, but both show similar half-lives. Sensitivity analysis showed that drug absorption is sensitive to effective permeability but not sensitive to particle radius and small intestinal transit time. The two products were found bio-inequivalent which is suggested to be due to differences in formulation additives that decreased the effective permeability of Flagyl.

Keywords: Intestinal permeability; Oral absorption; Metronidazole

1. Introduction

The oral bioavailability of an agent is affected by many factors including: dissolution, transit time, intestinal permeability, formulation additives and first pass metabolism in the gut and/or liver. Intestinal permeability, a key step in drug absorption, quantitates the fundamental transport property of the intestinal mucosa for a particular compound. The rate of permeation is dependent upon several factors including the structure and integrity of the intestinal membrane, the physiochemical properties of the drug, the specific transport mechanisms involved, and sometimes the inclusion of formulation additives. Intestinal permeability can be determined experimentally by different methods such as single-pass perfusion technique in situ and regional jejunal perfusion technique in vivo [1–6].

On the other hand, recently with more information available about gastrointestinal tract physiology, such as gastric emptying rates and mean residence times in different parts of the gut, and with more understanding of the variables affecting them [7,8], intestinal permeability can now be estimated indirectly from drug plasma profiles when drug pharmacokinetic and physicochemical properties are known. This can be achieved with the aid of new computer software especially designed for such purposes. This method is advantageous as it can provide estimates of intestinal permeability in all parts of the intestine without the need to perform numerous in vivo experiments.

In this paper, an experimental application of this recent approach is presented to investigate the oral rate and extent of absorption of two metronidazole suspension products. Metronidazole (1*H*-imidazole-1-ethanol 2-methyl-5-nitro) has long been used for treatment of anaerobic and microaerophilic microorganisms including *Entameba histolytica*, *Giardia lamblia*, and *Trachomonas vaginalis*. It is usually about 80% absorbed orally, but in some patients absorption is low [9].

2. Materials and methods

2.1. Drugs and reagents

Test product suspension of particle radius less than 40 μm was purchased from the Jordanian market. Flagyl suspension, Batch No. 382, of particle radius 40–75 μm was purchased from the Saudi market. All reagents used

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were obtained from Sigma Chemical Company, St. Louis, MO.

2.2. Subjects

Twenty-four healthy male subjects gave written informed consent to participate in the study. The study was approved by the Institutional Review Board of the study site, Ibn-Annafis hospital. The subjects were within 15% of their ideal body weight and were judged to be healthy based on medical history, physical examination, complete blood count and serum chemistry. In addition, all subjects were medication free, including over-the-counter agents, for 7 days prior to the study.

2.3. Experimental and assay procedure

Following a 10-h overnight fast, a 500-mg dose of metronidazole suspension was administered orally followed by 200 ml water in a two-phase, two-sequence crossover study with a 2-week washout period. Blood samples were collected at $0_{\text{pre-dose}}$, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 6, 8, 10, 14, 18, 24, 30 and 36 h after dosing. Samples were stored at −20°C until analyzed by a sensitive hplc method. The procedure involves extraction of metronidazole and the internal standard ornidazole from 0.5 ml plasma using 6 ml ethyl acetate. The mixture was then vortexed for 1 min and centrifuged for 5 min at 3000 rev./min. The organic layer was evaporated to dryness at 45°C under nitrogen. The residue was reconstituted in 200 μl mobile phase before injection into HPLC. The separation was performed at 318 nm using purospher column, 5 μm RP–18e, with a mobile phase of 65:35 v/v water-methanol at a flow rate of 1.5 ml/min. Hplc system consisted of LC-10AD pump and SPD-10A UV detector (Shimadzu, Japan). Limit of quantification was 0.1 μg/ml. Intra-day and inter-day coefficient of variations were less than 8%.

2.4. Data analysis

2.4.1. Disposition kinetics

Areas under plasma concentrations (AUC_{0-36},$AUC_{0-\text{infinity}}$), maximum concentration ($C_{\max}$), time to reach maximum concentration ($T_{\max}$), and elimination half life were calculated by non-compartmental analysis for all subjects using TOPFIT® software [10] after logarithmic transformation of the individual data.

2.4.2. Absorption kinetics

Metronidazole physicochemical parameters and human gastrointestinal physiological parameters were obtained from literature and from our pharmacokinetic analysis, and were used as baseline values for optimization, simulation and sensitivity analysis. The effective permeabilities at different parts in the gastrointestinal tract were optimized to match the mean plasma drug concentrations using Gastro-Plus® software [11]. Optimization was performed by the Optimization Module using a modified Hooke and Jeeves Pattern Search Method, which has been used widely in optimization software in aerospace industries. This was done by searching for the best permeability value in each part of the gut that produces plasma concentration according to Eq. (1) that matches the mean plasma concentration at the same time [11]

$$C(t) = (FDP_{\text{eff}})/(VR((P_{\text{eff}}/R) - (0.693/T_{0.5})) \times [e^{-(0.693/T_{0.5})*t} - e^{-(P_{\text{eff}}/R)*t}] \quad (1)$$

where (P_{eff}/R) is (effective permeability/intestinal radius) and equals the local absorption rate constant (K_a) at each part of the gut [12]; F is absolute bioavailability; D is oral dose; V is volume of distribution; and $T_{0.5}$ is half-life.

This is a more sophisticated way than a single compartment absorption model, as it accounts for the interactive time dependencies of absorption and the pharmacokinetic phenomena.

The optimized parameters were then used to calculate the fraction of oral dose absorbed (F_a) from each part of the gut using Eq. (4) by running single simulation mode. This was achieved by numerically solving Eqs. (2) and (3) simultaneously [13]. The system of differential equations is integrated using Runge–Kutta numerical integration package [11]

$$dr^*/dZ^* = -D_n\,(1-C^*)\,/3r^* \quad (2)$$

$$dC^*/dZ^* = D_n\,D_o\,r^*\,(1-C^*) - 2A_n\,C^* \quad (3)$$

$$F_a = 1 - \{r^*\}^3 - \{C^*/D_o\} \quad (4)$$

where A_n is an absorption dimensionless number equal to (P_{eff}/R) multiplied by mean transit time; D_o is a dose dimensionless number equal to (initial dose concentration)/drug solubility; D_n is a dissolution dimensionless number equal to $\{(\text{Diffusivity}/r_0)C_s(4\pi r_0^2)/((4/3)\pi r_0^3\ \text{density})\}/(Q/\pi R^2 L)$; r^* is the radius of particle/initial radius r_o; Z^* is equal to Z/L where L is intestinal length; C^* is equal to C_L/C_s where C_s is drug solubility; and Q is intestinal flow rate.

The human gut is divided into stomach with 0.25 h mean transit time, seven parts of small intestine each with mean transit time of 0.4714 h, and colon with 24 h mean transit time [11].

In addition, parameter sensitivity analysis was performed by running sensitivity analysis mode. This was done by calculating F_a using Eqs. (2–4) in which the studied parameter were changed while other parameters were kept constant at their baseline values. This allows filtration of parameters in our model that affect F_a largely from those that are not.

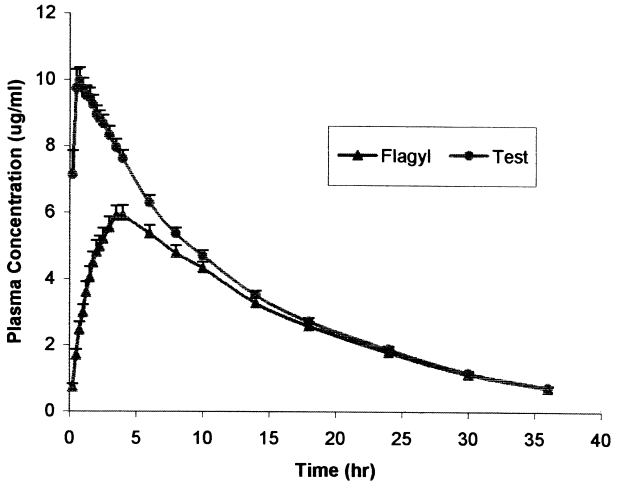

Fig. 1. Metronidazole mean plasma profiles (+SEM) for Flagyl and the test product

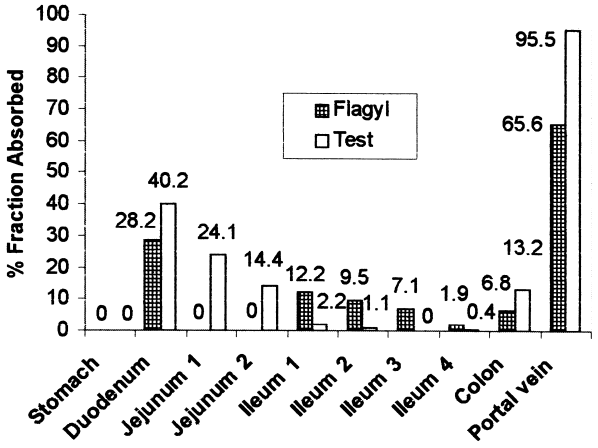

Fig. 2. Metronidazole compartmental absorption from the different parts of GIT

2.4.3. Statistical analysis

Analysis of variance for the disposition parameters, after logarithmic transformation of AUC_{0-36}, $AUC_{0-infinity}$ and C_{max}, and confidence limits for their ratios were done using SAS® software, GLM procedure [14].

3. Results

Mean plasma concentrations for the two metronidazole products are summarized in Fig. 1. Disposition and statistical analysis results are summarized in Table 1. The point estimates of test product to Flagyl ratio and analysis of variance were calculated after logarithmic transformation for the AUC_{0-36}, $AUC_{0-infinity}$ and C_{max} parameters. The optimized duodenal, jejunal1, jejunal2, illial1, ileal2, ilial3, ilial4 and colonic permeability values ($\times 10^{-4}$) for the test and Flagyl products were 3.96, 3.96, 3.96, 0.68, 0.37, 0.01, 0.12, 0.38 and 2.34, 0, 0, 1.2, 1.1, 0.9, 0.3, 0.04 cm/s, respectively. The absorbed fractions are summarized in Fig. 2. In addition, the sensitivity analysis results of metronidazole drug represented by Flagyl product is summarized in Fig. 3.

4. Discussion

All disposition kinetic parameters, except the half-life parameter, of the test product were significantly different from Flagyl parameters. The $AUC_{0-infinity}$ and C_{max} are 1.26 and 1.86 times more, respectively, in the test product. However, both showed similar half-life values. The two formulations were bio-inequivalent. It was assumed that absorption of the test product was significantly higher than Flagyl. Hence, the absorption kinetics were investigated in detail for both products to validate our assumption.

The optimized effective permeability values of the test product were higher than those of Flagyl, which supports the above assumption. We noticed that permeability values decrease as drug moves down towards the colon suggesting that higher parts of small intestine are the main sites for metronidazole. On the other hand, the fractions of oral dose absorbed from different parts in the gut were a function of permeability values and residence times in each part. Hence, higher fractions were absorbed from the colon as compared with each ilium part. This is due to a higher residence time of 24 h in the colon, as compared with the small intestinal residence time of 3.3 h. The total fraction of oral dose absorbed to the portal vein is 1.46 times more in the test product, which is consistent with the AUC's and C_{max} ratio results.

Table 1
Metronidazole pharmacokinetic and statistical analysis summary for Flagyl and the test product

Parameter	Flagyl (SD)	Test (SD)	Test/Flagyl % ratio (limits)	ANOVA *P*-value[a]
AUC_{0-36} (μg × h/ml)	101.1 (23.9)	127.3 (21.5)	128 (118–140)	<0.001
$AUC_{0-infinity}$ (μg × h/ml)	111.6 (28.3)	137.9 (25.5)	126 (115–138)	<0.001
C_{max} (ug × ml)	6.1 (1.5)	11.1 (2.1)	186 (166–210)	<0.001
T_{max} (h)	3.8 (0.9)	0.9 (0.6)	20 (16–26)	<0.001
Half life (h)	9.5 (1.9)	9.5 (1.7)	100 (93–108)	0.778

[a] Refer to *P*-values of the treatment source of variation in each parameter.

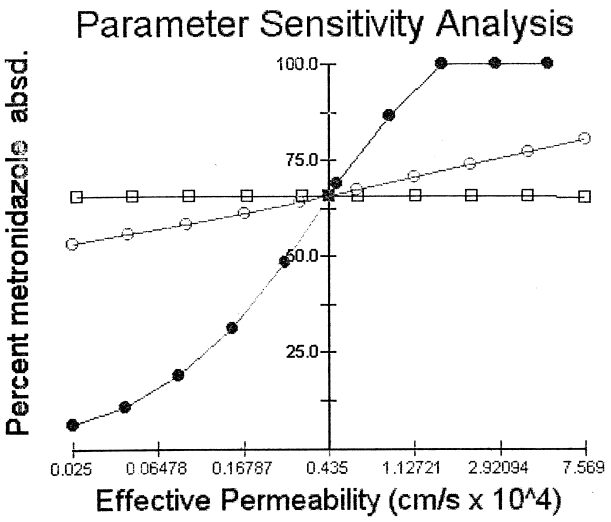

Fig. 3. Flagyl parameter sensitivity analysis. ○ small intestine transit with a range of 1.65–6.6 h, ● effective permeability, n particle radius with a range of 25–200 μm

Sensitivity analysis was then performed to investigate the effect of changing the particle radius by micronization, changing the effective permeability by using formulation additives, or changing the small intestinal transit time via other drugs. Metronidazole absorption was found insensitive to micronization, highly sensitive to changes in the effective permeability value, and slightly sensitive to changes in small intestinal transit time. The effect of micronization is expected because metronidazole can be classified as a highly soluble drug according the new biopharmaceutical drug classification [12]. The average dissolution times in the gut, based on drug water solubility of 10 mg/ml, were around 0.02 and 0.003 h for particle radii of 70 and 30 μm respectively. Thus, drug dissolution in vivo is not rate limiting its oral absorption. This was validated by sensitivity analysis results that showed model insensitivity to particle size changes. The formulation additives in Flagyl are suggested to decrease metronidazole oral absorption, which is advantageous, as it will render the drug more available to eradicate lower bowl amebas. However, the test product showed higher oral absorption and hence higher systemic bioavailability.

5. Conclusions

The test product exhibited significantly higher absorption rate and extent than Flagyl, but both show similar half-lives. The two products were found to be bio-inequivalent due to differences in formulation additives that decreased the effective permeability of Flagyl.

Pharmaceutical researchers are encouraged to incorporate absorption, metabolism and toxicity studies earlier in the discovery phase rather than waiting until the development phase. Such simulation studies can save drug companies time and money, because very often it is not the most potent compound that makes it to market. Rather, a less potent compound with better absorption, metabolism and toxicity characteristics will often be the market success.

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