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BIOAVAILABILITY OF MESALAZINE FROM TWO COATED FORMULATION TABLETS

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Abstract: HPLC methodology with a fluorescent detector is suitable for bioavailability studies of investigated generic tablets Mesalazine 250 mg (Jelfa, Poland) versus standard Salofalk tablets (Dr. Falk, Germany). The investigations were completed in ten healthy subjects in a double way crossover design. Only one bioavailability parameter – time for maximum mesalazine plasma concentration (t_{max}) and overall elimination rate constants are significantly greater for the above standard tablets. The parameters like maximum plasma drug concentration (C_{max}), lag time for absorption (T_{lag}), biological half-life time ($t_{1/2}$) are also more favorable for the standard, but these values are not significantly different.

Keywords: bioavailability, human subjects, 5-ASA matrix tablets, pharmacokinetic parameters, TopFit computer program, excipients, sustained release

Abbreviations: ML: mesalazine tablets (Jelfa), SL: salofalk tablets (dr. Falk), mesalazine: 5-aminosalicylic acid (5-ASA), 4-ASA: 4-aminosalicylic acid (the internal standard)

There are two major entities comprising inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD). The former is a chronic, idiopathic, nonspecific and diffuse inflammation of the large bowel mucosa and bloody diarrhea is the main sign of the disorder. In turn, if CD is also a chronic, idiopathic and nonspecific inflammatory process of the gastrointestinal tract (GI), the process is segmental and transmural, and it may develop in any part of the gut from the mouth to the anus. Diarrhea, abdominal pain, cramping, fever, and weight loss are the most common symptoms and signs of the disease. A fluctuating course with alternating periods of remission and relapses are typical for the natural history of IBD and the relapse is either mild or severe. Both entities occur mostly between the second and third decade of life. The diagnosis of IBD is suspected on the basis of the clinical picture and confirmed after careful physical examination followed with additional studies such as blood and stool tests, endoscopy, histopathology, and radiological methods. At least one-fifth of the patients with UC and majority with CD develop in time various complications, the presence of which often necessitates surgery. However, most of the patients with mild flares are treated symptomatically with a medical approach, and 5-ASA is the first line medication widely used in clinical practice (1). 5-ASA, also known as 5-aminosalicylic acid is an anti-inflammatory drug, with a primarily topical mechanism of action. It is possible that it recedes inflammation by blocking the cyclooxygenase pathway and inhibiting prostaglandin production in the gut. 5-ASA is administered for induction therapy as well as for maintenance therapy in IBD. There are various formulations of 5-ASA such as oral preparations which are indicated for inflammation within the small bowel and for right-sided colitis, enemas used in left-sided colitis and suppositories, indicated for proctosigmoiditis (2).

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As far as the oral preparations are considered, Asacol and Salofalk are indicated for an ileocolonic type of CD as well as for UC because of sustained release of 5-ASA within the distal ileum and colon. In turn, Olsalazine, Sulfasalazine, and Balsalazide should be administered exclusively in UC because the colon is the major site of delivery. Finally, Pentasa is an exceptional oral formulation of 5-ASA due to a slow and gradual release of the active moiety within the entire digestive tract from the duodenum to the colon. Therefore, the formulation is indicated for both inflammatory bowel disease with various sites of inflammation (3). The oral formulations prevent rapid and nearly complete systemic absorption of 5-ASA within the proximal jejunum. It is estimated that the total absorption of the dose reaches 20-30%. Afterward the absorbed 5-ASA is acetylated in the gut and by the liver, and the metabolites are excreted mainly with urine. Apart from that, these oral preparations delay the release of 5-ASA into the distal ileum or colon for 3-4 h, making the active moiety being available at the site of inflammation (4).

The aim of this work is to compare the bioavailability of 250 mg tablet formulations: Salofalk (Dr. Falk, Germany) as a standard, widely used in the medical treatment and an investigated, generic tablets – Mesalazine (Jelfa, Poland). The therapeutic requirement is that a tablet active substance should not be absorbed from the GI tract before approximately 3 h delay from its per-oral administration. The discussion is supposed to be also introduced on the onset of pharmaceutical excipients on the production of a matrix formulation in which the dissolution of an active substance is sustained.

EXPERIMENTAL

Methods

Pharmacokinetic analysis

The analytical results of the plasma specimens were used to calculate the following parameters: overall elimination rate constants: (K, h^{-1}), half-life time ($t_{1/2}$), area under the plasma concentration-time curve ($AUC_{0\rightarrow\infty}$, $mg\cdot(h\cdot l)^{-1}$, time to peak plasma concentration (t_{max},h), peak plasma concentration (C_{max}, $mg\cdot l$) and lag time of absorption (T_{lag}, h). TopFit 2.0 software (Gustav Fischer, Stuttgart, 1993) was used for calculation of the above pharmacokinetic parameters. Statistical significance of 5-ASA plasma variations of two formulation tablets was tested according to an ANOVA test by the Excel 2013 program.

Materials

Products samples identified as mesalazine coated tablets 250 mg (ML), lot number 10795p as well as salofalk coated tablets 250 mg (SL), lot number 93H25778 were received from Jelfa, Jelenia Góra, Poland and purchased from "Dr. Falk" , Germany, respectively. The salofalk tablets are widely used in medical treatments and therefore the following excipients are provided on a patient flyer: sodium carbonate exsiccated, glycine, povidone K25, microcrystalline cellulose, sodium carboxymethylcellulose, silica colloidal exsiccated, calcium stearate, hypromellose, macrogol 600, eudragit L 100 and F, titanium dioxide (E 171), ferrous(II) oxide yellow, (E 172). A 5-ASA authentic sample was obtained from Jelfa. An internal standard – 4-aminosalicylic acid – was purchased from Sigma-Aldrich, Germany. Methanol (Merck,

Table 1.Characteristics of volunteers.

Number of a volunteer	Initials	Age (years)	Body mass (kg)	Height (cm)
I	S.A.	21	69	180
II	P.W.	26	70	178
III	P.M.	22	59	168
IV	K.P.	30	74	178
V	S.A.	22	70	180
VI	L.A.	28	53	167
VII	B.K.	21	70	180
VIII	S.M.	35	90	177
IX	W.P.	22	84	180
X	S.J.	32	74	178
Means	-	25.9	71.3	176.6
SD	-	4.8	10.1	4.7

Table 2. Crossover tablets administration.

Number of a volunteer	Order of tablet kind administration	
	A (ML)	B (SL)
I	A	B
II	A	B
III	A	B
IV	A	B
V	A	B
VI	B	A
VII	B	A
VIII	B	A
IX	B	A
X	B	A

Germany), tetrabutylammonium hydrogen sulfate(VI) (Sigma-Aldrich, Germany), sodium hydrogen phosphate(V) anhydrous (Fluka Chemie AG, Switzerland) were of HPLC grade. Potassium dihydrogen phosphate(V) (Xenon, Łódź, Poland) was of reagent grade. Water was three times house distilled from silica equipment.

Apparatus

An Isochrom Spectra-Physics chromatograph with a fluorescent detector and an integrator (San Jose, CA, USA) were coupled to a 10 µm ODS reverse phase column (4.6 x 250 mm). The analytical column was protected by a guard column (40 x 4.6 mm ID), both obtained from Merck, Germany. The fluorescence intensity of eluent was monitored at 330 nm of excitation maximum and 510 nm of emission maximum.

Mobile phase

A mixture of methanol-phosphate buffer pH 7.5-0.1% tetrabutylammonium hydrogen sulfate (240 : 760 : 1, v/v) was filtered through a 0.45 µm nylon membrane filter and deaerated. The above buffer was prepared of KH_2PO_4 and Na_2HPO_4 aqueous solutions, both of 0.05 M concentration. The mobile phase was pumped at a rate of 1 mL/min at room temperature.

Stock solutions and standards

Stock solutions containing 0.2, 0.5, 1, 2, 5, 10, 20 and 40 g/L 5-ASA were prepared in double distilled water. Fifty µL of each standard was transferred to a culture tube containing 0.5 mL of plasma, 50 µL of double distilled water and 0.5 mL of 0.2 g/L methanol 4-ASA solution (internal standard). The tubes were closed with PTTE lined screw cap and vortex-mixed for 2 min., stored for 0.5 h at 4°, and centrifuged at 600 g for 5 min. The resulting plasma-based standards containing 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 and 4 mg/L 5-ASA and 5 mg/L 4-ASA were processed according to the procedure described below.

Procedure

Twenty µL of clear supernatant was injected directly onto the column. Peak area ratio of 5-ASA to 4-ASA was plotted versus 5-ASA concentration in mg/L and the resulting calibration curve was used to calculate the serum concentration of unknown samples. Calibration curves were made on three days of three independent series. Each series consisted of 8 separate samples.

Subject selection

Ten each of normal adult, non-smoking, male and female volunteers between 20 and 36 years (mean 25.9 ± 4.8) weighing on average 71.3 ± 10.1 kg of height 176.6 ± 4.7 cm were selected for participation in the above investigation (Table 1). The volunteer subjects were selected after completing a thorough history and physical examination and after a normal laboratory examination. The laboratory test consisted of the following: hematology, serum chemistry, and urinalysis. All subjects were presented with full details of the investigation, both verbally and in written form, prior to providing written informed consent. Furthermore, the volunteers did not use any other drugs and alcohol within 24 h before the experiments and during their course. The

investigations were approved by the Human Investigations Ethical Committee at Poznań University of Medical Sciences.

Study design

The study was of a non-blinded, open-label, single dose, double way crossover design. All subjects were randomly assigned a drug assignment number from I to X, which was used throughout the period (Table 2). Dosing periods were separated by at least a 7 days washout period. The subjects were required to fast for at least 10 h prior to the timing of the next tablet administration. On each of two treatment days, subjects were instructed to present in the

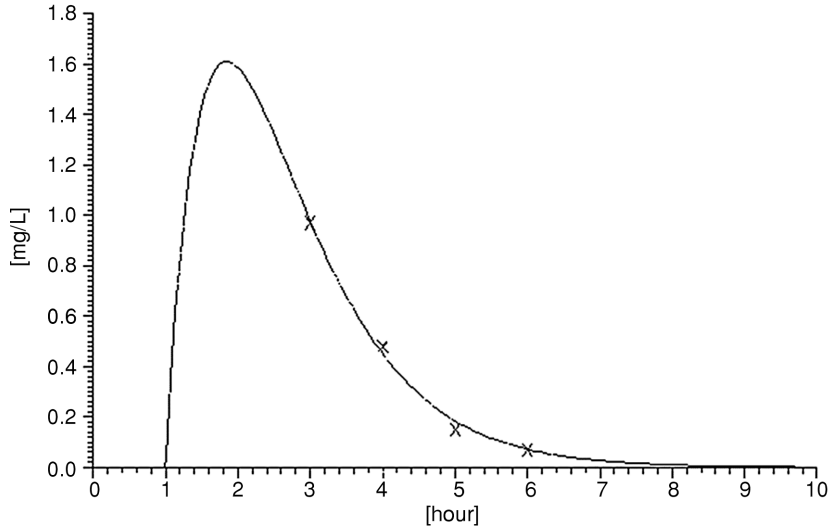

Figure 1. Mean plasma 5-ASA concentrations (mg/L) as a function of time (h) after the crossover administration of a single 250 mg mesalazine (ML) tablet (Jelfa) to 10 volunteers simulated by a computer program TopFit 2.0 (Gustav Fischer, Stuttgart, 1993)

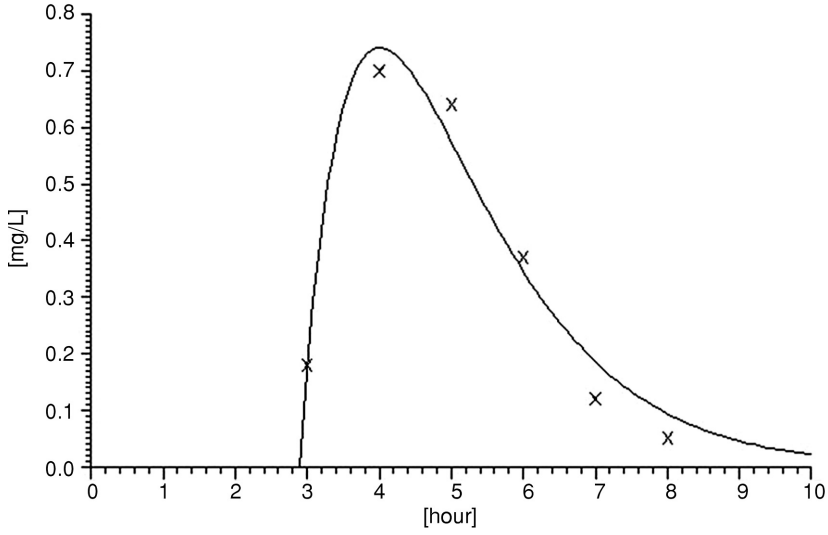

Figure 2. Mean plasma 5-ASA concentrations (mg/L) as a function of time (t) after the crossover administration of a single 250 mg salofalk (SL) tablet (dr. Falk) to 10 volunteers simulated by a computer program TopFit 2.0 (Gustav Fischer, Stuttgart, 1993)

Table 3. Plasma concentrations of 5-ASA (mg/L) for 10 subjects (I - X) after administration of a single dose 250 mg ML tablet.

Time [h]	I	II	III	IV	V	VI	VII	VIII	IX	X
3	1.48	1.20	0.85	0.97	0.56	0.96	1.41	1.07	1.23	< 0.02
4	0.72	0.39	0.37	0.32	0.18	0.35	0.42	0.39	0.90	0.76
5	0.32	0.12	0.09	0.06	0.06	0.10	0.20	0.12	0.15	0.28
6	0.14	0.06	0.04	0.02	0.02	0.03	0.12	0.02	0.05	0.17
7	0.05	< 0.02	0.02	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	0.03
8	0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
10	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	0.02	< 0.02

Table 4. Plasma concentrations of 5-ASA (mg/L) for 10 subjects (I - X) after administration of a single dose 250 mg SL tablet.

Time [h]	I	II	III	IV	V	VI	VII	VIII	IX	X
3	0.59	< 0.02	0.12	< 0.02	< 0.02	0.78	0.08	< 0.02	< 0.02	0.22
4	1.58	0.14	0.93	0.44	0.24	1.77	0.59	0.28	0.80	0.27
5	0.75	0.12	0.62	0.23	0.60	0.35	1.32	0.76	1.56	0.11
6	0.34	0.09	0.68	0.06	0.78	0.10	0.96	0.38	0.32	0.03
7	0.10	0.05	0.15	0.04	0.53	0.02	0.20	0.07	0.04	< 0.02
8	0.02	< 0.02	0.06	0.02	0.31	< 0.02	0.06	0.02	0.03	< 0.02
10	< 0.02	< 0.02	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

Table 5. Mean plasma concentrations of 5-ASA (mg/L) (±SD) from 10 subjects after the crossover single dose administration of ML and SL tablets and suitable pharmacokinetic parameters.

Time [h]	Mesalazine (ML)	Salofalk [SL]	Statistics (ANOVA) $\alpha = 0.05$
3	0.97 ± 0.44	0.18 ± 0.28	p < 0.001
4	0.48 ± 0.23	0.70 ± 0.57	NS
5	0.15 ± 0.09	0.64 ± 0.49	p < 0.006
6	0.07 ± 0.06	0.37 ± 0.33	p < 0.009
7	< 0.02	0.12 ± 0.16	-NS
8	< 0.02	0.05 ± 0.09	-NS
10	< 0.02	< 0.02	NS-
Pharmacokinetic Parameters			
AUC [(mg/(L h)$^{-1}$]	2.02 ± 0.94	2.48 ± 1.29	NS
t_{max} [h]	2.78 ± 0.47	4.04 ± 0.59	p < 0001
C_{max} [mg/L]	1.83 ± 0.90	1.17 ± 0.84	NS
T_{lag} [h]	1.598 ± 1.232	2.743 ± 0.618	NS
K [h^{-1}]	1.283 ± 0,280	0.962 ± 0.414	p < 0.001
$t_{1/2}$ [h]	0.564 ± 0.118	0.891 ± 0.544	NS

study facility. At zero hour the subjects were assigned to a phlebotomist for the purpose of collecting a 5 mL blood sample. The assigned tablet was swallowed with 200 mL water. All subjects abstained from food until the 4-h blood specimen was obtained when a standardized low-fat lunch was provided. Regular meals were resumed after a 10-h blood sample was obtained. Following drug administration, venous blood samples (5 mL) were obtained (in Serum Gel tubes, S/4.7 mL, Sarstedt Monovette, Germany) from the subject's right or left antecubital fossa at the following times: immediately before administration of drug studied and 3, 4, 5, 6, 7, 8 and 10 h after administration. Within 30 min following blood withdrawal, the samples were centrifuged. The separated plasma samples were frozen in plastic vials at -20^{o} and labeled with the subjects I.D. number, the drug assignment numbers, treatment day and time of sampling. The red blood cells were discarded.

RESULTS AND DISCUSSION

Analytical methodology

Calibration curves of peak height ratio versus concentration were linear over the concentration range 0.02-4 mg/L and the intercept was essentially zero. The correlation coefficient for 24 independent determinations was 0.9998 whose coefficient of variation in % was 0.77. The intercept was –0.003 ± 0.027 and significantly not different from zero. The linear calibration curve equation was $A_{5\text{-ASA}}/A_{4\text{-ASA}}$, where $A_{5\text{-ASA}}$ and $A_{4\text{-ASA}}$ are an area under the peak of 5-ASA and 4-ASA (internal standard), respectively calculated by means of the above integrator, and C is the concentration of 5-ASA.

Pharmacokinetic analysis

The serum 5-ASA concentrations (C) following the administration of a single tablet (ML or SL) (Tables 3 and 4) absorbed and eliminated according to the first order processes in an open one-compartment body model were well characterized by the difference in two exponentials (Figs. 1 and 2):

$$C = B \cdot e^{-\lambda_2 \cdot t} - A \cdot e^{-\lambda_1 \cdot t}$$

where A and B are the corresponding zero-time intercepts, λ_1 and λ_2 are the apparent first-order fast and slow disposition rate constants, and t is the time. The individual and averaged 5-ASA plasma data and their S.E.M.s, as well as the pharmacokinetic parameters, are given in Tables 3-5 for both tablet formulations.

The mean data for both formulations are presented in Figures 1 and 2.

The so-called sustaining effect of the absorption phase is observed caused by the excipients: coating of tablets to prevent their disintegration in the stomach juice and an addition of e.g. kollidon SR, eudragit L (methacrylic acid as a tablet coating material in the case of salofalk), ethylcellulose (Pentasa, coated microgranules) (4, 5) to promote biological action in distal part of the GI tract. Kollidon SR is a physical mixture of 80% hydrophobic poly(vinylacetate), 19% hydrophilic povidone (kollidon 30, poly(vinyll pyrrolidone)), 0.8% sodium lauryl sulfate and 0.2% colloidal silicon dioxide. As an effect of the above sustaining formulation agents, the absorption phase of pharmacokinetic curves is observed in minority and therefore the elimination phase dominates (Figs. 1 and 2). The sustaining effect of the absorption is more significant in the case of SL tablets. It is characterized in its suitable pharmacokinetic parameters: longer T_{lag} (2.74 h) and t_{max} (4.04 h) times and lower C_{max} (1.17 mg/L) concentration if compared to corresponding parameters of ML tablets: 1.6, 2.78, 1.83, respectively (Table 5). The absorption phase was negligible and mainly observed before 3 h have elapsed. Therefore, the first order absorption rate constants could not be calculated with sufficient precision. However, the TopFit computer system provides their values, which are not included in that paper. Those values are close to the elimination rate constants. Usually, the absorption rate constants are much greater than the elimination rate constants. The phenomenon observed, is of course, the result of the release-retarding formulation excipients, which were necessary to provide a pharmacological effect in the distal part of the ileum. It is also favorable for SL tablets that their elimination rate constants are significantly lower if compared to ML tablets (Table 5).

The terminal phase of 5-ASA pharmacokinetic curve fulfills the monexponential equation (Figs. 1 and 2), because absorption in the proximal part of the small bowel is negligible:

$$C' = C_{max} \cdot e^{-K \cdot t},$$

where C' is 5-ASA concentration at time when the absorption can be neglected, K is the 5-ASA overall elimination rate constants and t is the time when the absorption can be neglected (usually over 3 h). A semi-logarithmic equation of the above elimination phase is linear:

$$\ln C' = \ln C_{max} - K \cdot t,$$

The slope of the above equation gives us the first order overall elimination rate constants (Table 5). Biological half-life time is calculated from the equation characteristic for first-order processes:

$$t_{1/2} = \ln 2/K$$

These values are as it follows: 0.564 and 0.89 h^{-1}, respectively (Table 5). They are not different from the literature data (4), which are between 0.5 – 2.4 h.

Comparison of the above formulations let us state that both tablets represent coated oral 5-aminosalicylic acid formulation of retarded absorption. Standard salofalk tablets are a better choice for treatment of ulcerative colitis, because lag time for absorption is longer (2.74 vs. 1.60 h) and their t_{max} also is more favorable because is longer (4.04 vs 2.78 h, $p < 0.001$) as well as their maximum concentration in blood is lower (1.17 vs. 1.86 mg/L) which however are insignificant (Table 5). It is also true for C_{max} values (1.17 vs. 1.83).

CONCLUSIONS

Mesalazine 250 mg investigated, generic tablets (Jelfa, Poland) are slightly less favorable versus standard Salofalk 250 tablets (Dr. Falk, Germany) as coated 5-ASA intra-intestinal tablets because their time for maximum blood concentration (t_{max}) is significantly shorter and overall elimination rate constant is significantly greater than for the above standard. However, the other bioavailability parameters: AUC, T_{lag} and C_{max} are insignificantly statistically different.

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REFERENCES

1. Sairenji T., Collins K.L., Evans D.V.: Prim. Care 44, 673 (2017).
2. Abraham D.P., Ahmed T., Ali T.: Handb. Exp. Pharmacol. 239, 115 (2017).
3. Criscuoli V., Modesto I., Orlando A., Cottone M.: Expert. Opin. Pharmacother. 14, 1669 (2013).
4. Sandborn W.J., Hanauer S.B.:. Aliment. Pharmacol. Ther. 17, 29 (2003);
5. Hermann T.W., Dobrucki R., Piechocki S., Resztak M., Reh R.: Med. Sci. Monit. 11, 181 (2005).

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