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Pharmacokinetics and Safety of a New 1200 mg Single-Dose Delayed Release Mesalazine Microgranule Formulation

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Abstract

The treatment of Inflammatory Bowel Diseases (IBDs) requires a relative high therapeutic daily dose of mesalazine and thus, the drug formulation need to be well tolerate and safe.

A new pH-dependent controlled release 5-ASA microgranule formulation in 1.2 g sachets has been developed. The plasma levels of both the active principle 5-ASA and the main metabolite N-Acetyl-5-ASA, after oral administration of the new formulation or after an equimolar dose of three separated enteric coated 400 mg tablets administered in the same time (Pentacol® 400, SOFAR, Milan, ITALY), were measured with a validated high performance liquid chromatography-tandem mass spectrometry method. C_{max}, t_{max} and AUC values were considered as primary variables and the drug safety was the secondary one.

The plasma 5-ASA concentration appearance was faster after microgranule administration (tmax of 8.1 hours) than after the reference tablets assumption (tmax of 10.6 hours). The Cmax and AUC values were similar for both formulations and the kinetic of plasma disappearance of the test formulation was slight faster. The inter-subject variability was lower after administration of the microgranules with a %CV of 17.5% vs 40.4% for the tablets (n=23), due to a more controlled homogeneous drug release from the granule format. The N-Acetyl-5-ASA metabolite presents a similar plasma profile of the 5-ASA for both formulations. The use of microgranules is safe and will allow to reduce the daily dosages, by improving the patients compliance also in presence of difficulty to swallow large tablets.

Keywords: IBD; Mesalazine; 5-ASA; Inflammation; Microgranules; Pharmacokinetics

Introduction

Inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis (UC), are chronic inflammatory disorders of the gut which ethiopathogenesis is still poorly understood.

5-aminosalicylic acid (5-ASA) is an anti-inflammatory drug widely used for the treatment of IBDs (Akobeng et al., 2005; Baumgart et al., 2005; Klein et al., 2005; Nielsen et al., 2007; Ransford et al., 2002; Sutherland et al., 2006a, 2006b).

5-ASA is a bowel-specific drug that is metabolized in the gut and has its predominant local action with a poor intestinal absorption and bioavailability and few systemic side effects. In the colonic epithelium, 5-ASA undergoes to an extensive metabolism by the enzyme N-acetyltransferase I to form N-Acetyl-5-ASA (N-Ac-5-ASA) (Sandborn et al., 2004). This compound, considered therapeutically inert (van Hogezand et al, 1988), is the major metabolite present in the

blood reaching levels similar to the 5-ASA. Usually, plasma concentration of N-Ac-5-ASA is also measured to more accurately evaluate the systemic exposure to 5-ASA and the pharmacokinetics of the drug (Sandborn et al., 2004; Tjørnelund et al., 1991; Wilding et al., 2003).

5-ASA acts topically reducing the inflammation in the intestinal mucosa promoting the healing of the epithelium (Baumgart et al., 2005).

The drug oral formulations are designed to pass through the stomach and the small intestine without being released, metabolized and absorbed, so the active ingredient is delivered directly to the site of inflammation in the large intestine (Prakash et al., 1999; Sciarretta et al., 1993).

Usually, the oral delayed-release 5-ASA is an enteric-coated formulation which releases the active ingredient bolus in the terminal ileum and colon by pH-dependent dissolution mechanism (Klein et al., 2005).

Different micropellet and microgranule formulations of 1 and 1.5 g of 5-ASA have been recently described with similar pharmacokinetics to the conventional tablets (Wilding et al., 2003; Brunner et al., 2003; Readler et al., 2004; Wiersma et al., 2004).

Up to 74% of patients with mild to moderately active ulcerative colitis experience endoscopic or symptomatic improvement (including remission) or both when treated with oral delayed-release 5-ASA at a related high dosage of 2.4-4.8 g/day.

Moreover, the results of plasma pharmacokinetic analyses of delayed-release formulations of 5-ASA have shown high inter-patient variability, mainly because of the low bioavailability of 5-ASA and a poor drug release optimization. For a drug like 5-ASA, the plasma concentration levels are not fully relevant to evaluate the pharmacological activity since only the local drug concentration is important. In addition, previous studies demonstrated a correlation between plasma 5-ASA profile and the kinetic of the drug release from the coating formulation; thus, plasma profiles are important to evaluate the time of the release while the C_{max} is important for the safety evaluation (Lichtenstein et al., 2008).

In the present paper, pharmacokinetic data of a new slow-release 5-ASA formulation are reported. The formulation (sachets containing 1.2 g 5-ASA in enteric coated microgranules) has been designed to improve and optimize the 5-ASA pharmacokinetic and activity by reducing the daily dosage regiments.

The new formulation is composed by 5-ASA microgranules coated with anionic copolymers of Eudragit S100 and Eudragit L, which ensure a complete release of the active ingredient at pH 7.5. The drug release kinetic and efficiency have been optimized not only for the pH-dependency but also for the coating layer properties in term of size and wetability in order to minimize inter-subject differences in intestinal behaviour and colonic segmental transit time.

Preliminary data on plasma 5-ASA and N-Ac-5-ASA concentration-time profiles, obtained after administration of 1.2 g of 5-ASA as 3 tablets of 400 mg each, using an HPLC-ESI-MS/MS method developed in our laboratory (Pastorini et al., 2008), were in agreement with those reported by other authors for similar formulations and in proportion to the dosages administered (e.g. 0.8 and 1.5 g) (Sandborn et al., 2004; Wilding et al., 2003). The developed analytical method is accurate, precise, selective and sensitive enough to allow the analysis of 5-ASA and N-Ac-5-ASA as low as 0.2 ml of human plasma collected during clinical studies in which 5-ASA drugs are administered at therapeutic dosage.

The pharmacokinetic (PK) concentration profiles of plasma 5-ASA and its metabolite N-Ac-5-ASA, obtained after a single dose oral administration of the new formulation have been evaluated in 23 healthy volunteers and compared with those obtained after administration of 3 x 400 mg enteric coated commercially available tablets (Pentacol® 400, SOFAR, Milan).

The safety of the new formulation was evaluated not only by the PK parameters but also by performing biochemical tests before and after each treatment (including haematocrit, urinalysis and liver function test) and performing at each blood sampling clinical evaluations by monitoring blood pressure, cardiac frequency, breath rate, body temperature and body weight.

Material and Methods

Study Objective

The main objective of the study was to evaluate the PK of a new enteric coated microgranule oral formulation with pH-dependent controlled release in 1.2 g sachets. Safety and pharmacokinetic parameters have been evaluated by comparison with commercially available enteric coated 400 mg tablets (3x400 mg) with pH-dependent controlled release (reference formulation: Pentacol® 400, SOFAR, Milan).

Plasma levels of both the unmodified active principle 5-ASA and the main metabolite N-acetyl-5-ASA, after oral administration of an equivalent single dose of the two formulations (1200 mg), have been evaluated.

Microgranule Formulation

The test formulation is made of mesalazine microgranules (1.2 g), resistant to the gastric pH and with a pH-dependent release. The formulation is composed of mesalazine as active principle and Vivapur 12, polyethylene glycol 400, Aerosil 200, hydroxypropyl methylcellulose 615, Endragit S100, Eudragit L100-55 and trietyl citrate, as eccipients for the delayed release properties.

The in vitro drug dissolution kinetic test was performed in accordance with the guidelines of the US Pharmacopeia. The percent release of the active principle from the reference formulation was 0% at pH 1 after 2 h and 70%±5%at pH 7.5 after 45 minutes. The percent release of the drug from the test formulation was 0% at pH 1 after 2 h, and 78%±3 at pH 7.5 after 45 minutes. Then, dissolution test data showed that in vitro 5-ASA dissolution rate for microgranule formulation is slightly higher than for the tablets.

Design of the Stydy

This was a single-centre, single dose, randomized, open labelled, cross-over study in healthy volunteers. The study was divided in two periods: in the first period the subjects were randomly treated with the formulation A (1 microgranules sachet containing 5-ASA 1.2 g) or with the formulation B (3 tablets containing 5-ASA 400 mg each). The period lasted 48 h during which the subjects underwent blood sample collection. After further 48-h washout, all subjects underwent the second 48-h period, during which the subjects previously administered the treatment A were given the treatment B, and vice versa. The total duration of the trial was 7 days.

Selection of Study Population

Healthy volunteers were selected as study population. Subjects included both male and female aged between 18 and 60 years, Caucasian, with a Body Mass Index (BMI) >19 and < 28, a normal physical examination, a normal routine haematology, blood chemistry and urinalysis, and who were willing to participate to the trial by providing written informed consent.

Subjects were excluded who met the following criteria: acute or chronic illness, positive history of clinically significant abnormalities, physical examination positive for clinically significant abnormalities, clinically significant abnormalities of any routine haematology, blood chemistry or urinalysis parameters, history of exposure to risk of HIV infection, history of alcohol or drug abuse, tabagism (> 10 cigarettes/ day), drug intake within the previous 4 weeks, intake during the study of lactulose, or other products that, by decreasing colonic pH, could impede the 5-ASA release by the test and reference formulations, clinically significant history of hypersensitivity to active ingredients or drugs, pregnant women, participation in other clinical studies during the previous 6 months, over 600 ml blood loss during the previous 3 months, inability or not willing to comply with the protocol procedures. Subjects prematurely discontinuing the trial could not be admitted again.

The study was approved by the State authority and the Institutional Ethics Committee (S. Orsola-Malpighi Hospital, University of Bologna, Italy). The study was conducted in accordance with the current revision of the Declaration of Helsinki concerning medical research in human and with current Good Clinical and Laboratory Practice Guidelines.

25 subjects (9 females and 16 males, mean age 26.6±3.3) were screened and 24 subjects were randomized because one subject in the sequence A-B dropped out from the study for consent withdrawn. 13 subjects were randomly assigned to sequence 1: A-B (microgranules in 1.2 g sachets –3 X 400 mg tablets) and 11 to sequence 2: B-A (3x400 mg tablets – microgranules in 1.2 g sachets).

As regards sex, subjects were equally distributed in the two sequence groups. The mean age in the two sequence groups was similar, 26 years and 28 years respectively. No female subjects underwent pregnancy test because they declared to use adequate method of contraception.

The average treatment compliance was about 96% in sequence A-B group and 100% in group B-A. One subject of A-B group took the treatment only in one period and then withdrew consent (subject was excluded from any analysis): excluding him the compliance was 100% in both groups. The final number of subjects who completed the study was 23.

Study Medication

The reference formulation was three tablets of Pentacol® 400 mg (SOFAR, Milan) administered at once with water for a total dose of 1.2 g 5-ASA. The studied new formulation was one sachet of microgranules of 1.2 g 5-ASA, administered in 250 ml of commercial apricot fruit juice to favour the suspension of granules. The dose was in the range of those approved for Pentacol®. The product was administered to the subjects by the study doctor at the starting day of each treatment sequence.

Subjects enrolled in the study had a fast time starting from the midnight of the night before the drug assumption until lunch. No coffee, smoke, chocolate and other prokinetic foods were allowed during the study. The meal allowed during

all the period of the study was a standard meal (breakfast with yogurt or cereals - 400Kcal; lunch with 80 g of pasta, 120 g of meat or 200 g of fish, 50 g of bread - 1000 Kcal; snack with yogurt - 150 Kcal; dinner with 80 g of pasta, 60 g of cheese - 900 Kcal).

All subjects underwent 8 ml blood sampling at the following time points: 0 (immediately before drug intake), 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h, 32 h and 48 h post-dose. The samples were centrifuged at 2000 x *g* for 10 min. The resulting plasma fraction was frozen as 3 separate aliquots at –80°C until the analysis.

Safety and Tolerability

During screening period the following assessment was carried out: physical examination, complete blood cell count with white blood count (WBC) differential, erythrocyte sedimentation rate (ESR), reactive protein C (RCP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glutamyl transpeptidase (GGT), serum creatinine, total bilirubin, alkaline phosphatase (AP), glucose, blood urea nitrogen (BUN), electrolytes, urinalysis, vital signs (systolic and diastolic blood pressure, heart rate, breath rate, body temperature, body weight). Vital signs were measured at each blood sampling. All the other examinations were performed during screening and at the end of each treatment period of the crossover design. All adverse events (AEs) and serious AEs were carefully monitored.

HPLC-ESI-MS/MS Method for 5-ASA and N-Ac-5-ASA Analysys in Plasma Samples

5-ASA and its main metabolite N-Acetyl-5-ASA were determined in plasma by a new method based on high performance liquid chromatography-tandem mass spectrometry with electrospray source (HPLC-ESI-MS/MS) developed and validated in our laboratory (Pastorini et al., 2008) according to the ICH-GLP guidelines. Briefly, 490 µl of plasma were spiked with 10 µl of the internal standard working solution to achieve final concentrations of 100 and 400 ng/ml of 4-ASA and N-Acetyl-4-ASA, respectively. 1 ml of methanol was added to the mixture to achieve protein precipitation. Sample were stirred and centrifuged at 12000 x *g* for 10 min. Then, 1.2 ml of the surnatant was transferred into plastic vials and dried under vacuum. The residue was dissolved in 500 µl of 50 mM acetic acid. Finally, 4 µl of this solution was injected into the HPLC-ESI-MS/MS system for the analysis. The two analytes were separated using a C18 column (Synergi Hydro-RP, 4µm, 150 mm x 2.0 mm i.d.) with a mobile phase composed of 17.5 mM acetic acid (pH 3.3):acetonitrile=85:15 (v/v) at 0.2 ml/min flow rate under isocratic elution conditions. Selective detection was performed by tandem mass spectrometry with electrospray source, operating in negative ionization mode and in multiple reaction monitoring acquisition (m/z 152 → 108 for 5-ASA; m/z 194 → 150 and 194 → 107 for N-Ac-5-ASA).

Pharmacokinetic and Statistical Analysis

5-ASA and N-Ac-5-ASA concentrations found in plasma were processed by means of a non compartmental PK analysis to obtain the following parameters: C_{max}, maximum concentration; t_{max}, time to maximum concentration; AUC_{0-t}, area under concentration-time curve, from 0 to the last blood sampling time point with measurable concentration; $AUC_{0-\infty}$, area under concentration-time curve, from 0 to the infinity; $t_{1/2}$, elimination half life, calculated as 0.693/ß.

The following populations were defined: safety population as all randomized subjects who took at least one dose of the study drug in at least one of the two study periods, Intention To Treat (ITT) population as all randomized subjects who took the study drug in each of the two study periods and for whom the pharmacokinetic evaluations were available and Per Protocol (PP) population as all randomized subjects who took the study drug and completed both the treatment periods without major protocol violations. All the analyses were performed on the data obtained from the subjects who completed entirely the study protocol. Consequently, the primary population for the pharmacokinetic analyses was the PP population while the analyses done on ITT and safety populations are to be considered as supportive.

The primary variables, such as the C_{max} and the AUC_{0-t} after log transformation, were analyzed by means of an analysis of variance (ANOVA) model for crossover design: the effects of sequence, subjects within sequence, period and treatment were included in the model as fixed effects. The comparison of pharmacokinetic parameters obtained for the two formulations was performed by means of Schuirmann's two one-sided 't' test. As recommended by "Guidance for Industry-Statistical Approaches to Establishing Bioequivalence" (FDA 2001), the 90% confidence limit of the rate between the two formulations means was calculated; this is equivalent to carrying out two one-sided tests of hypothesis at 5% level of significance (Schuirmann, 1987). Statistical analysis was performed by means of the SAS software for Windows Version 8.2 (SAS Institute); pharmacokinetic analysis was performed by means of Equivtest/PK Version 2.0 (Statistical Solutions).

Results and Discussion

HPLC-ESI-MS/MS Method

The used HPLC-ESI-MS/MS method fulfilled all the

requirements for an accurate and a precise analysis of 5-ASA and N-Acetyl-5-ASA. When the analytical performances were compared with previously reported and used methods, this one offers undoubted advantages in term of simple pre-analytical treatment not requiring derivatization and in terms of overall analytical performance, allowing to identify and quantify accurately the two analytes with higher selectivity and sensitivity.

The HPLC-ESI-MS/MS method for determination of 5-ASA and N-Ac-5-ASA was validated fulfilling the acceptance criteria generally established for bioanalytical assays when applied in pharmaceutical analysis. For both analytes the limit of detection (LOD) and the limit of quantification (LOQ) were 15 ng/ml and 50 ng/ml respectively, matrix-matched standard curves showed linearity up to 4000 ng/ml (weighting factor 1/x).

The selected internal standards, 4-ASA and N-Ac-4-ASA, which are position isomers of analyte, presented a similar MS/MS behaviour in term of ionization efficiency and were well separated from the corresponding analytes. In the entire analytical range the within- and between-batch precision (CV%) values were respectively =6.3% and =11% for 5-ASA and =8.0% and =10% for N-Acetyl-5-ASA. For both analytes the within- and between-batch accuracy (bias%) values ranged respectively from -8.4% to 7.9% and from -7.9% to 8.0%. The overall recoveries (n=6) at three tested concentration levels (i.e., 100, 1000 and 4000 ng/ml) were respectively > 90% for 5-ASA and > 95% for N-Acetyl-5-ASA (RSD% = 10%). The selectivity of the method was verified against endogenous matrix components. The stability of 5-ASA and its metabolite in human plasma was verified after 3 freeze/thaw cycles, after storage at room temperature for 4 hours and in extract after storage in autosampler plate at +7°C for 42 hours. The long-term stability of 5-ASA and N-Ac-5-ASA in human plasma stored at -80°C was observed until 4 months.

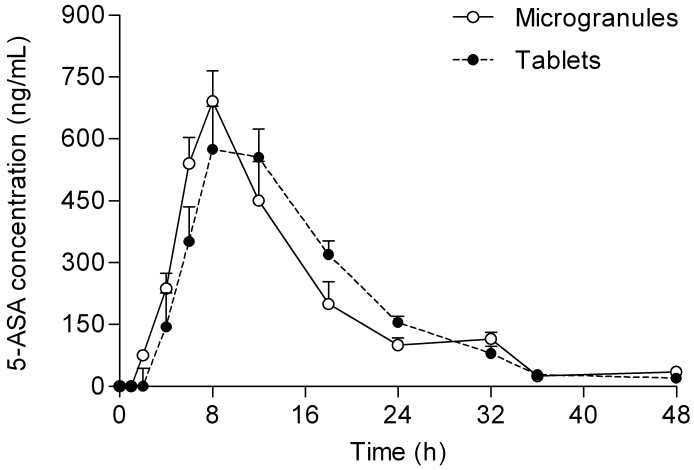

Figure 1: Mean plasma 5-ASA concentration-time profiles following a single oral dose administration in healthy volunteers (n=23, PP population). Standard errors of the mean (S.E.M) are also reported.

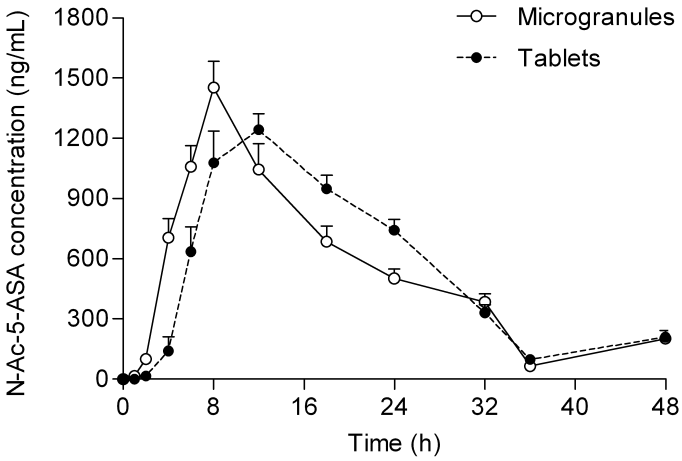

Figure 2: Mean plasma N-Ac-5-ASA concentration-time profiles following a single oral dose administration in healthy volunteers (n=23, PP population). Standard errors of the mean (S.E.M) are also reported.

Plasma Concentrations and Pharmacokinetic Evaluation

The mean 5-ASA concentration time-profiles for the two studied formulations are reported in Figure 1. The kinetic profiles were slightly different in the two formulations, being the average peak values around 8 h after microgranules intake and slightly delayed (at 10 h), after tablets intake. The rate of absorption of the drug from the enteric coated formulation, as indicated by the plasma concentration kinetic, was faster for microgranules than for tablets; and after 4 h the plasma concentration was twofold higher in comparison with the concentration reached after the tablet administration. The significant faster kinetic of 5-ASA appearance in the blood after granule administration is probably due to the physical state of the formulation composed by microgranules with a consistent increased surface area available for the solubilisation process (Brunner et al., 2003). For both formulations, a similar C_{max} was obtained, suggesting that a similar amount of 5-ASA is located in the intestine. After the $t_{ma,x}$ the kinetic of the microgranules was faster and slightly lower plasma levels were obtained 24 h after the administration.

Figure 2 shows the mean of N-Acetyl-5-ASA concentrations in the two formulation groups over the time in the PP population (all the results are reported in Table 2). The N-Ac-5-ASA plasma kinetic profiles were also quite

		Microgranules (n=23)	Tablets (n=23)
C_{max}	Mean (± S.D.) (ng/ml)	795 (± 363)	835 (± 557)
	Min-Max (mg/ml)	265-1350	240-1480
	Coefficient of Variation (%)	45.6	66.7
	Geometric Mean (ng/ml)	700	689
t_{max}	Mean (± S.D.) (h)	8.1 (± 1.4)	10.6 (± 4.3)
	Min-Max (h)	6.0-12.0	6.0-18.0
	Coefficient of Variation (%)	17.5	40.4
	Geometric Mean (h)	8.0	9.9
AUC_{0-t}	Mean (± SD) (ng.h/ml)	9060 (± 4170)	9180 (± 4940)
	Min-Max (ng.h/ml)	3570-23120	3490-27340
	Coefficient of Variation (%)	46.1	53.9
	Geometric Mean (ng.h/ml)	8070	8070
$AUC_{0-\infty}$	Mean (± S.D.) (ng.h/ml)	9490 (± 4120)	9380 (± 4950)
	Min-Max (ng.h/ml)	4250-22040	4370-25990
	Coefficient of Variation (%)	43.4	52.7
	Geometric Mean (ng.h/ml)	8550	8260
$t_{1/2}$	Mean (± S.D.) (h)	8.0 (± 6.0)	6.0 (± 3.8)
	Min-Max (h)	2.3-18.2	2.4-15.3
	Coefficient of Variation (%)	74.9	63.9
	Geometric Mean (h)	6.5	5.1

Table 1: Summary statistics of 5-aminosalicylic acid (5-ASA) pharmacokinetic parameters by treatment (PP population). C_{max}, peak concentration of 5-ASA; t_{max}, time to peak concentration of 5-ASA; AUC_{0-t}, area under the plasma concentration vs. time curve up to time t at which the last detectable concentration of 5-ASA were observed; $AUC_{0-\infty}$, area under the plasma concentration vs. time curve up to infinity; $t_{½}$, elimination half-life.

		Microgranules (n=23)	Tablets (n=23)
C_{max}	Mean (± S.D.) (ng/ml)	1554 (± 612)	1471 (± 585)
	Min-Max (mg/ml)	762-3290	802-3110
	Coefficient of Variation (%)	39.4	39.8
	Geometric Mean (ng/ml)	1460	1378
t_{max}	Mean (± S.D.) (h)	8.0 (± 1.5)	11.5 (± 4.5)
	Min-Max (h)	6.0-12.0	6.0-24.0
	Coefficient of Variation (%)	18.5	39.4
	Geometric Mean (h)	7.9	10.7
AUC_{0-t}	Mean (± S.D.) (ng.h/ml)	28120 (± 7820)	28770 (± 9770)
	Min-Max (ng.h/ml)	13430-48170	13550-56650
	Coefficient of Variation (%)	27.8	34.0
	Geometric Mean (ng.h/ml)	27030	27246
$AUC_{0-\infty}$	Mean (± S.D.) (ng.h/ml)	33190 (± 8830)	34500 (± 13730)
	Min-Max (ng.h/ml)	15270-50980	13550-74600
	Coefficient of Variation (%)	26.6	39.8
	Geometric Mean (ng.h/ml)	31950	32060
$t_{1/2}$	Mean (± S.D.) (h)	15.6 (± 7.2)	14.2 (± 6.9)
	Min-Max (h)	2.4-36.1	2.4-24.8
	Coefficient of Variation (%)	46.5	48.4
	Geometric Mean (h)	13.9	12.2

Table 2: Summary statistics of N-Acetyl-5-ASA (N-Ac-5-ASA) pharmacokinetics parameters by treatment (PP population). C_{max}, peak concentration of N-Ac-5-ASA; t_{max}, time to peak concentration of N-Ac-5-ASA; AUC_{0-t}, area under the plasma concentration vs. time curve up to time t at which the last detectable concentration of N-Ac-5-ASA were observed; $AUC_{0-\infty}$, area under the plasma concentration vs. time curve up to infinity; $t_{½}$, elimination half-life.

similar for both treatments: after the tablets administration the t_{max} was slightly longer and the kinetic slower when compared to the microgranule administration.

The mean AUC_{0-t} values were similar for both formulations (Tables 1-2) and confirmed by the Schuirmann's 't' test. For 5-ASA, the 90% confidence interval of the antilogged difference of the means of the formulations was 0.8048-1.2430, included in the equivalence range planned in the protocol (0.80, 1.25). Schuirmann's two one-sided 't' test reject both the null-hypotheses of non-equivalence with p-values 0.0457 and 0.0460, respectively. For N-Ac-5-ASA the 90% IC on AUC(0-t) of the antilogged difference of the means of the formulations was 0.8802-1.1177, included in the equivalence range planned in the protocol (0.80, 1.25). Schuirmann's two one-sided t test rejected both the null-hypotheses of non-equivalence with p-values 0.0027 and 0.0015, respectively.

Calculated mean Cmax values were similar for both formulations (Tables 1-2), as confirmed by the Schuirmann's 't' test. For 5-ASA, the 90% confidence interval of the antilogged difference of the means of the formulations was 0.7867-1.3127, included in the equivalence range planned in the protocol (0.70, 1.43). Schuirmann's two one-sided 't' test rejected both the null-hypotheses of non-equivalence with p-values equal to 0.0102 and 0.0160, respectively.

For N-Ac-5-ASA 90%IC of antilogged difference of the Cmax means of the formulations was 0.9419-1.1927; Schuirmann's two one-sided t test rejected both the null-hypotheses of non-equivalence with p-values equal to <0.0001 and 0.0001, respectively. The C_{max} values agreed with those previously measured after administration of 5-ASA if the different doses used were normalized (Sandborn et al., 2004; Wilding et al., 2003). Data obtained indicated that the intestinal absorption and the consequent plasma levels of the drug presented an higher variability in the subjects assuming three independent tablets containing 400 mg each compared with the microgranules formulated for a single dose administration (Tables 1 and 2).

Safety and Tolerability

During screening period all the subjects presented normal values obtained by biochemical function tests and physical examinations. Both biochemical tests and vital signs remained unchanged during the study, proving that they were not affected by the treatments.

Conclusions

Our results demonstrate that the test formulation is safe and has a similar pharmacokinetic compared to the reference one. Furthermore, microgranule administration results in a faster appearance of the drug in blood in respect to the tablets (reference formulation) due to a faster gastric release. In fact, microgranules are released following the liquid-phase content that is faster than the solid content release which involves considerably larger size table (Brunner et al., 2003).

Due to the poor systemic bioavailability of the 5-ASA, plasma pharmacokinetic does not predict accurately the 5-ASA concentration at the colonic mucosa level. Indeed, 5-ASA should be considered as a topical product. This pharmacokinetic study has been used mainly to exclude high plasma concentration after the high single dose administration and in general to exclude potential toxicity of the drug. In fact, if the systemic bioavailability of the test formulation is not different from that of the reference, it can be assumed that the systemic safety profile of the test formulation is identical to that of the reference formulation (Readler et al. 2004). Moreover, the similar pharmacokinetic behaviour obtained for the test and the reference formulations will allow a simplified further clinical development by entering directly in phase III studies, without need of dose-finding studies for the test formulation. It could also be indicative of a potential therapeutic equivalence between the two formulations according to the FDA recommendations.

In addition, a slow release formulation will allow a reduced number of daily dosages where a daily dosage of 4.8-5 g is needed, as well as in paediatric and geriatric patients, who have a rather higher difficulty to swallow large size tablets (Ng et al., 2008).

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References

1. Ahnfelt-Rønne I, Nielsen OH, Christensen A, Langholz E, Binder V, et al. (1990) Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. Gastroenterology 98: 1162-1169.» Pubmed » Google Scholar

2. Akobeng AK, Gardener E (2005) Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's Disease. Cochrane Database Syst Rev 1, CD003715. » CrossRef » Pubmed » Google Scholar

3. Baumgart DC, Vierziger K, Sturm A, Wiedenmann B, Dignass AU (2005) Mesalamine promotes intestinal epithelial wound healing in vitro through a TGF-beta-independent mechanism. Scand J Gastroenterol 40: 958-964. » CrossRef » Pubmed » Google Scholar

4. Brunner M, Greinwald R, Kletter K, Kvaternik H, Corrado ME, et al. (2003) Gastrointestinal transit and release of 5-aminosalicylic acid from 153Sm-labelles Mesalazine pellet vs tablets in male healthy volunteers. Aliment Pharmacol Ther 17: 1163-9 64. » CrossRef » Pubmed » Google Scholar
5. FDA, Center for Drug Evaluation and Research (CDER) (2001) Guidance for Industry- Statistical Approaches to Establishing Bioequivalence, www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf
6. Gionchetti P, Guarnieri C, Campieri M, Belluzzi A, Brignola C, et al. (1991) Scavenger effect of sulfasalazine, 5-aminosalicylic acid, and olsalazine on superoxide radical generation. Dig Dis Sci 36: 174-178. » CrossRef » Pubmed » Google Scholar
7. Klein S, Stein J, Dressman J (2005) Site-specific delivery of anti-inflammatory drugs in the gastrointestinal tract: an in-vitro release model. J Pharm Pharmacol 57: 709-719. » CrossRef » Pubmed » Google Scholar
8. Klotz U, Stracciari GL (1993) Steady state disposition of 5-aminosalicylic acid following oral dosing. Arzneimittelforschung 43: 1357-1359. » CrossRef » Pubmed » Google Scholar
9. Lichtenstein GR, Kamm MA (2008) Review article: 5-aminosalicylate formulations for the treatment of ulcerative colitis - methods of comparing release rates and delivery of 5-aminosalicylate to the colonic mucosa. Aliment Pharmacol Ther 28: 663-673. » CrossRef » Pubmed » Google Scholar
10. Myers B, Evans DN, Rhodes J, Evans BK, Hughes BR, et al. (1987) Metabolism and urinary excretion of 5-aminosalicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract. Gut 28: 196-200. » CrossRef » Pubmed » Google Scholar
11. Nielsen OH, Munck LK (2007) Drug insight: aminosalicylates for the treatment of IBD. Nat Clin Pract Gastroenterol Hepatol 4: 160-170. » CrossRef » Pubmed » Google Scholar
12. Ng SC, et al. (2008) Review article: new drug formulations, chemical and therapeutic approached for the management of ulcerative colitis. Alimentary Pharmacol Ther 28: 815-29» CrossRef » Pubmed » Google Scholar
13. Pastorini E, Locatelli M, Simoni P, Roda G, Roda E, et al. (2008) Development and validation of a HPLC-ESI-MS/MS method for the determination of 5-aminosalicylic acid and its major metabolite N-acetyl-5-aminosalicylic acid in human plasma. J Chromatogr B 872: 99-106. » CrossRef » Pubmed » Google Scholar
14. Prakash A, Markham A (1999) Oral delayed-release mesalazine: a review of its use in ulcerative colitis and Crohn's disease. Drugs 57: 383-408. » Pubmed » Google Scholar
15. Ransford RAJ, Langman MJS (2002) Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. Gut 51: 536-539. » CrossRef » Pubmed » Google Scholar
16. Readler A, Behrens C, Bias P (2004) Mesalazine (5 -aminosalycilyc acid)micropellets show similar efficacy and tolerability to Mesalazine tablets in patients with ulcerative colitis results from randomized-controlled trial. Aliment Pharmacol Ther 20: 1353-63. » CrossRef » Pubmed » Google Scholar
17. Sandborn WJ, Hanauer SB, Buch A (2004) Comparative pharmacokinetics of equimolar doses of 5-aminosalicylate administered as oral mesalamine (Asacol) and balsalazide: a randomized, single-dose, crossover study in healthy volunteers. Aliment Pharmacol Ther 19: 1089-1098.» CrossRef » Pubmed » Google Scholar
18. Schuirmann DJ (1987) A Comparison of the Two One-Sided Tests Procedure and the Power Approach for Assessing the Equivalence of Average Bioavailability. J Pharmacokinet Biopharm 15: 657-680.» CrossRef » Pubmed » Google Scholar
19. Sciarretta G, Furno A, Mazzoni M, Ferrieri A, Malaguti P (1993) Scintigraphic study of gastrointestinal transit and disintegration sites of mesalazine tablets labeled with Technetium-99m. Scand J Gastroenterol 28: 783-5. » CrossRef » Pubmed » Google Scholar
20. Sutherland L, MacDonald JK (2006a) Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev 2: CD000543. » CrossRef » Pubmed » Google Scholar
21. Sutherland L, MacDonald JK (2006b) Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev 2: CD000544.
22. Tjørnelund J, Hansen SH (1991) High-performance liquid chromatographic assay of 5-aminosalicylic acid (5-ASA) and its metabolites N-beta-D-glucopyranosyl-5-ASA, N-acetyl-5-ASA, N-formyl-5-ASA and N-butyryl-5-ASA in biological fluids. J Chromatogr 570: 109-117.» CrossRef » Pubmed » Google Scholar
23. van Hogezand RA, van Hees PA, van Gorp JP, van Lier HJ, Bakker JH, et al. (1988) Double-blind comparison of 5-aminosalicylic acid and acetyl-5-aminosalicylic acid suppositories in patients with idiopathic proctitis. Aliment Pharmacol Ther 2: 33-40.» CrossRef » Pubmed » Google Scholar
24. Wilding IR, Behrens C, Tardif SJ, Wray H, Bias P, et al. (2003) Combined scintigraphic and pharmacokinetic investigation of enteric-coated mesalazine micropellets in healthy subjects. Aliment Pharmacol Ther 17: 1153-1162. » CrossRef » Pubmed » Google Scholar