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A comparative bioavailability study of a generic capsule formulation containing carbocysteine

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The bioequivalence of two carbocysteine capsulae preparations was assessed in 18 healthy volunteers who received a single 750 mg dose of each carbocysteine formulation, and a new sensitive method for the quantification of carbocysteine in human plasma was developed. The study was conducted using an open, randomized, two-sequence, two-period crossover design with a week washout period between the succesive treatments. Plasma samples were obtained over a 12-hour period and analyzed by high performance liquid chromatography coupled to electrospray ionization-mass spectrometry. Either a multiplicative statistic model for concentration-dependent parameters or an additive approach for time-related parameters were used for the comparison of pharmacokinetic parameters describing both the early and total exposure to carbocysteine. The respective 90% confidence limits [CL] of the individual ratios of geometric means were 0.898 to 1.112 [point estimate 0.999] for C_{max} and 0.923 to 1.210 [point estimate 1.057] for $AUC^{0-\infty}$, while the difference between times elapsed to reach C_{max} was insignificant [$p = 0.4497$]. Since both 90% CL for the log-transformed $AUC^{0-\infty}$ and C_{max} geometric mean ratios were included in the proposed 0.80–1.25 interval, test drug (Bronchobos® capsules) was considered bioequivalent to the reference one (Mucopront® capsules).

1. Introduction

Carbocysteine and its lysine salt are derivatives of the amino acid cysteine and have mucolytic and anti-inflammatory properties. It is effective for the prevention of exacerbation of chronic bronchitis (Allegra et al. 1996), but has no role in the treatment of an acute respiratory attack. The drug is also used as an adjunct to standard treatment of inflammation in otorhinolaryngology (Cattabeni et al. 1990).

Therapeutic inadequacy, in general, could arise beyond therapeutic switching from the innovator to generic drug formulation due to its insufficient bioavailability. To avoid such a risk, careful pharmacokinetic studies are desired and suggested in the pre-registration period of generic preparations, including carbocysteine. Simultaneously, higher availability may result in an increased toxicity. In the case of carbocysteine this is less likely because it is a drug that has a wide therapeutic window (range of adult daily doses 900–3000 mg, pediatric doses range from 62.5 mg four times a day to 750 mg three times a day) and does not lead to serious effects in humans even in cases of huge overdoses.

Nevertheless, the purpose of this study was to evaluate the tolerability, pharmacokinetic properties and bioavailability/bioequivalence of carbocysteine capsules of the same strenght (375 mg) that are manufactured by two different pharmaceutical factories.

2. Investigations, results and discussion

Prior to initiation of the clinical part of the pharmacokinetic study the *in vitro* dissolution profiles of Bronchobos® capsules and Mucopront® capsules were compared. Clinical investigation was performed afterwards as a randomized, crossover trial on healthy volunteers. To determine the content of carbocysteine in plasma a validated HPLC method, combined with the electrospray ionization-mass spectrometry [HPLC-ESI-MS], was applied.

In a pH 4.0 buffered medium, at the temperature of 37 ± 0.5 °C, drugs under investigation dissolved between 81.2% (range 78.9–83.1%, Bronchobos) and 93.3% (range 90.4–98.0%, Mucopront). Both preparations, therefore, fulfilled the acceptance criteria that minimally 60% of the parent drug (Q) had to be released within 30 min (internal specification of the manufacturer Bosnalijek d.d., Bosnia & Herzegovina). Close releasing profiles of Mucopront and Bronchobos had indicated their pharmaceutical similarity and gave the opportunity of initiating a clinical pharmacokinetic trial.

A total of 18 subjects, whose demographic characteristics are summarized in Table 1, were selected to participate in the study. There were no differences in their age, weight or height that might compromise the validity of the planned pharmacokinetic trial.

Due to the lack of experimental data that document the large intra- and inter-subject pharmacokinetic variability of

Table 1: Demographic data of subjects (n = 18)

	Age (years)	Weight (kg)	Height (cm)
Mean	36.6	69.7	171.9
SD	11.7	12.4	8.7
Min.	21	50	155
Max.	53	100	183
CV (%)	32.1	17.8	5.0
Males, n = 8		Females, n = 10	

SD – standard deviation, CV – coefficient of variation

carbocysteine in humans, 18 subjects were arbitrarily chosen. That number was above the smallest sample (n = 12) that is accepted as sufficient to assess bioequivalence (Herchuelz 1996; Patnaik et al. 1996) and might represent a reliable number of participants for the bioequivalence decision. A post-study calculation based on the log-ANOVA error data (C_{max} CV-intra = 18.79%) revealed a sample size of 15 subjects to be quite enough to show a difference of 20% between the C_{max} values of the test and the reference articles (Diletti et al. 1992). The type I and type II errors would not exceed 5% and 20%, respectively.

Under the assay conditions described, linearity was observed in plasma standard curves of carbocysteine over a range of 0.25–40 μg/ml with a correlation coefficient greater than 0.999. The lower limit of quantification (LOQ) of carbocysteine was 0.25 μg/ml of plasma. Concentrations below LOQ were reported as 0.0 μg/ml. The mean recovery of extraction of carbocysteine from plasma samples amounted to 73.5%. For within-day analysis, the coefficient of variation of quality control samples (accuracy) ranged from −11% to +7.1% and the deviation (RSD) from expected concentration of carbocysteine, as a measure of precision, varied between 0% and 0.5%. For between-day analysis, the coefficients of variation were all within 15% and the corresponding RSD values ranged from 0.7% to 3.2%.

Analysis of the assay data indicated that the chosen HPLC-ESI-MS method was highly precise and accurate for performing a valid bioequivalence study. Its sensitivity was similar to that reported by Anacardio et al. (1997). However, the method was more sensitive than the HPLC technique with fluorescent, electrochemical and ultraviolet detection or the GC/GLC methodology (Maynard et al. 1978; Lutz et al. 1985; Bron 1986, 1988; De Schutter et al. 1988; Karim et al. 1988; Staffeldt et al. 1991).

The mean plasma concentration-time profile of carbocysteine is shown in the Fig. No marked differences were observed between the amounts of drug that had been absorbed from the formulations under the study. The tolerability of both preparations containing carbocysteine was reported as excellent.

The relevant pharmacokinetic parameters of carbocysteine for each preparation are listed in Table 2. As can be seen, the residual area (relation between AUC^{0-12} and $AUC^{0-\infty}$) of carbocysteine (2.9% Bronchobos, 2.5% Mucopront) accounted for less than 20% of the area from time 0 to time of the last measurable concentration. Therefore, it had satisfied the stated criterion ($AUC^{0-12}/AUC^{0-\infty} * 100 > 80\%$) and had no sizeable impact on the calculation of $AUC^{0-\infty}$ and, thus, on bioavailability.

The early exposure to carbocysteine, expressed through the values of peak concentrations and times to reach the peak, as well as the elimination half-lives, were of the same magnitude and, thus, comparable after application of

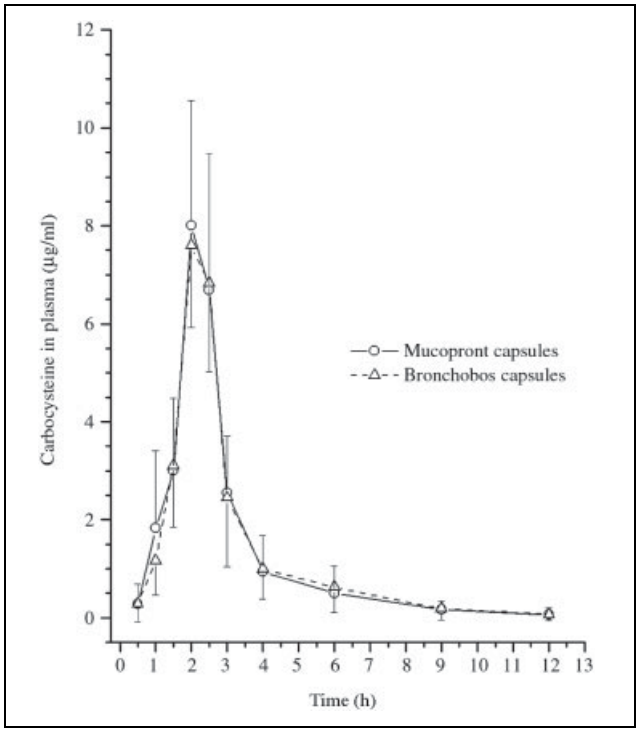

Fig.: Mean (±SD) concentration vs. time profiles of carbocysteine in plasma after the oral administration of a 750-mg dose of either Mucopront capsules or Bronchobos capsules to 18 healthy volunteers

Table 2: Mean values of pharmacokinetic parameters of carbocysteine after the ingestion of capsules Bronchobos and capsules Mucopront (dose 750 mg)

Parameter	Formulation					
	Mucopront (Reference)			Bronchobos (Test)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
C_{max} (μg · ml^{-1})	8.27	2.69	32.5	8.02	1.68	21.0
t_{max} (h)	2.08	0.19	9.1	2.17	0.24	11.1
k_e (h^{-1})	0.3976	0.1283	32.3	0.4070	0.1602	39.4
$t_{1/2}$ (h)	1.90	0.55	28.9	2.02	0.93	46.0
AUC^{0-12} (μg · h · ml^{-1})	14.36	6.57	45.8	14.39	5.08	35.3
$AUC^{0-\infty}$ (μg · h · ml^{-1})	14.82	6.99	29.0	14.88	5.42	38.3
Residual area (%)	2.5	2.8	112.0	2.9	3.5	120.7
MRT (h)	3.0	0.7	23.3	3.3	0.8	24.2

SD – standard deviation, CV – coefficient of variation

Table 3: Bioequivalence of carbocysteine from the Bronchobos 375 mg capsules versus Mucopront 375 mg capsules

Carbocysteine		C_{max}	$AUC^{0-\infty}$	t_{max}
Bronchobos	(Mean)	8.02	14.88	2.0*
375 mg carbocysteine	(Range)	6.11–10.75	7.86–26.97	2.0–2.5
Mucopront	(Mean)	8.27	14.82	2.0*
375 mg carbocysteine	(Range)	3.73–13.64	4.11–28.44	2.0–2.5
Point estimate of the ratio of the geometric means		0.999	1.057	–
90% confidence interval of the ratio of the geometric means		0.898 – 1.112	0.923 – 1.210	–
Wilcoxon signed ranks test		–	–	$p = 0.4497$
Acceptance range (bioequivalence)		0.80 - 1.25	0.80 - 1.25	$p > 0.05$
Conclusion		Bioequivalent	Bioequivalent	No significant difference

* Median value

the two carbocysteine-containing preparations. Slight differences (p, one-sided = 0.1958, n = 18) in the mean residence time (Bronchobos capsules = 3.3 h, Mucopront capsules = 3.0 h) were probably artefactual as the plasma concentrations at the end of the terminal phase had been low, variable and near the LOQ, resulting in relative imprecise estimations of this parameter.

The overall pharmacokinetic profile of carbocysteine in the present study was very close and in agreement with the data previously published (Maynard et al. 1978; Aiache et al. 1982; Lutz et al. 1985; Bron 1988). On the basis of maximum serum concentrations and $AUC^{0-\infty}$ values of carbocysteine it was concluded that they had not been significantly different between the studied preparations, with the power (derived from ANOVA) of 0.968 and 0.853, respectively.

The statistical evaluation of the main pharmacokinetic variables, which completely describe both the early and total exposure to carbocysteine, is presented in Table 3. The respective point estimates of the ratios of geometric means of C_{max} and $AUC^{0-\infty}$ of carbocysteine were 0.999 and 1.057 with 90% confidence intervals of 0.898–1.112 and 0.923–1.210, respectively. For the median t_{max} values, at a 5% level of significance, there were no significant differences between the formulations in the study as revealed by the non-parametric Wilcoxon signed ranks test.

Both 90% confidence intervals for $AUC^{0-\infty}$ and C_{max} geometric mean ratios were included in the 80% to 125% interval proposed by the U.S. Food and Drug Administration (FDA, 2000, 2001) and the difference between t_{max} values was statistically insignificant. On that basis Bronchobos capsules were considered bioequivalent to Mucopront capsules according to the early and the total exposure to carbocysteine. So, from the pharmacokinetic point of view studied drugs could be marked as interchangeable by a physician and might not be expected to produce therapeutic failure after switching the patient from one to another.

3. Experimental

3.1. Dissolution test

The *in vitro* dissolution rate in a pH 4.0 buffered medium for each dosage form was determined according to the general method of USP XXIV, rotating basket method (50 rpm; replication = 6). The test was performed using the Pharma Test (Model PTWS 3CE, Germany) dissolution apparatus.

The amount of dissolved drug, at time intervals 0, 10, 15, 20, 25 and 30 min, was determined spectrophotometrically (λ_{max} = 220 nm).

3.2. Clinical part

A single-dose, open-label, randomized, two-sequence, two-period crossover study design was used to evaluate the bioavailability of carbocysteine, prepared as 375-mg capsules by the manufacturers Bosnalijek d.d., Bosnia and Herzegovina (Bronchobos) and Heinrich Mack Nachf. GmbH & Co. KG, Germany (Mucopront). Eighteen subjects of either sex in good physical condition, as determined by complete medical and laboratory examinations before the study, were enrolled and provided written informed consent prior to any study related procedure. The study was approved by the Drug Commission and the Ethics Committee of the Military Medical Academy, Belgrade, Serbia and Montenegro on May 15, 2003.

The enrolled volunteers were randomly assigned to one of the two sequence groups such that upon completion of the study each subject received all two regimens. Dosing in each of the two consecutive periods was separated by a 1-week washout period. A single dose of 750 mg carbocysteine (two capsules) was given with 200 ml of non-carbonated mineral water following an overnight fast of at least 10 h.

The EDTA stabilized venous blood samples (approx. 8 ml) were collected prior to dosing (hour 0) and afterwards at time-points 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9 and 12 h. The samples were centifuged within one hour of collection and the plasma was separated and frozen at −20 °C until assayed.

3.3. Assay method

The analytical set (Waters Corporation, USA) was equipped with a liquid chromatograph Waters-Alliance 2695 Separations Module (with refrigerating autosampler, HPLC quaternary pump, column heater and degasser), mass spectrometer with electrospray ionization source (Waters-Micromass ZQ 2000 LC/MS – ESI System), computer IBM NetVista, Waters analytical column Spherisorb S10 SAX (particle size 10 μm), 4.6 mm × 250 mm (the column temperature adjusted to 20 °C), with Waters-SAX guard column (5 μm) in line holder type, and with a variable injector loop volume of 100 μl.

The HPLC-ESI-MS assay was performed by using mobile phase consisting of 1.55% formic acid in demineralized water, previously filtered and degassed by a membrane degasser. The flow rate through the column was 0.21 ml/min (splitted from 1 ml/min of the main flow). Mass quadropole detector was adjusted in SIM mode. A protonized positive molecular ion mass (m/z) for carbocysteine was 180 AMU throughout the assay. Under these conditions the retention time of carbocysteine was from 3.8 to 4.2 min. All the chemicals were of HPLC and p.a. purity and had been purchased commercially.

After melting to the room temperature to 1 ml of plasma sample 0.025 ml of formic acid was added. Vortex mixing for 30 s agitated the mixture; 1 ml of acetonitrile was added and further vortexed for 30 s. After 10 min 1 ml of mobile phase was added and again vortexed for 30 s. Thereafter, the sample was centrifuged for 10 min (3000 rpm) and the obtained supernatant was filtrated through a 0.2 μm PTFE filter. A final volume of 50 μl was injected into the HPLC-MS system and analyzed using the validated analytical method. Standard solutions (range of concentrations 0.25–40.0 μg/ml) and spiked plasma samples (range of concentrations 0.25–40.0 μg/ml) were also determined under the same assay conditions.

3.4. Pharmacokinetic analysis

The relevant pharmacokinetic parameters of carbocysteine were estimated using a noncompartmental technique. The peak plasma concentration (C_{max}) and the time elapsed to peak concentration (t_{max}) were obtained directly from the data. The elimination rate constant (k_e) was obtained from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. Half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$. The area under the carbocysteine plasma concentration-time curve (AUC^{0-12}) was computed using the linear trapezoidal rule while the area under the plasma concentration-time curve from time 0 to the infinite time ($AUC^{0-\infty}$) was calculated as the sum of AUC^{0-12} and C_t/k_e, where t was the time of the last measurable concentration (C_t) and k_e was the elimination rate constant.

MRT (mean residence time) was defined as the ratio of AUMC, the area under the first moment curve, and AUC (MRT = AUMC/AUC). The first moment curve is the area under the curve of the product of concentration and time ($C_p(t) \cdot (t)$] vs. time on a linear scale.

Parameters C_{max}, t_{max} and $AUC^{0-\infty}$ were accepted as the main variables, while AUC^{0-12}, residual areas ($AUC^{0-12}/AUC^{0-\infty} \cdot 100$), k_e, $t_{1/2}$ and MRT values served as the secondary pharmacokinetic objectives.

3.5. Statistical analysis

The following main pharmacokinetic parameters, which completely describe both the early and total exposure to carbocysteine, were subjected to statistical analysis: C_{max}, $AUC^{0-\infty}$ and t_{max}. The comparison of secondary parameters was only descriptive.

Following logarithmic transformation of $AUC^{0-\infty}$ and C_{max}, the values were subjected to analysis of variance (ANOVA) including terms for subjects, treatment (sequence) and period. For evaluation of bioequivalence the point estimates and 90% confidence intervals for the difference between test and reference formulations were constructed using the residual mean square error, obtained from the multifactorial ANOVA. The point estimates and the 90% confidence intervals were then back transformed to give estimates of the ratio of the geometric means and the corresponding 90% confidence intervals for the ratios of the two formulations in the comparison. A non-parametric test (Wilcoxon Signed Rank's Test) was performed for t_{max}.

Bioequivalence between the two formulations was accepted if the back transformed 90% confidence intervals for the geometric mean ratios od $AUC^{0-\infty}$ and C_{max} had follen within 0.80–1.25 range (FDA 2000, 2001; Hauck et al. 2001) and if the differences in t_{max} between the two formulations had been not statistically different.

References

Aiache JM et al. (1982) Comparative bioavailability of *S*-carboxymethylcysteine from two dosage forms: hard gelatin capsule and syrup. Biopharm Drug Dispos 3: 275–281.

Allegra L, Cordaro CI, Grassi C (1996) Prevention of acute exacerbations of chronic obstructive bronchitis with carbocysteine lysine salt monohydrate: a multicenter, double-blind, placebo-controlled trial. Respiration 63: 174–180.

Anacardio R et al. (1997) Quantification of *S*-carboxymethyl-(*R*)-cysteine in human plasma by high-performance ion-exchange liquid chromatography/atmospheric pressure ionization mass spectrometry. J Mass Spectrom 32: 388–394.

Bron J (1986) High pressure liquid chromatographic determination of carbocisteine in human plasma and urine. Pharm Weekbl Sci 8: 134–138.

Bron J (1988) Relative bioavailability of carbocysteine from three dosage forms, investigated in healthy volunteers. Biopharm Drug Dispos 9: 97–111.

Cattabeni F et al. (1990) Diffusione tessutale della S-cabossimetil-cisteina sale di lisina a livello della mucosa dell'orecchio e dei seni paranasali. Otorinolaringologia 40: 63–66.

De Schutter JA et al. (1988) Determination of S-carboxymethylcysteine in serum by reversed-pahse ion-pair liquid chromatography with column switching following pre-column derivatization with o-phthalaldehyde. J Chromatogr 428: 301–310.

Diletti E et al. (1992) Sample size determination for bioequivalence assessment by means of confidence intervals. Int J Clin Pharmacol Ther Toxicol 30: S51–58.

FDA (2000) Guidance for Industry. Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations. Avaliable at: http://www.fda.gov/cder/guidance/index.htm.

FDA (2001) Guidance for Industry. Statistical Approaches to Establishing Bioequivalence. Avaliable at: http://www.fda.gov/cder/guidance/index.htm.

Hauck WW et al. (2001) Limits of 80%–125% for AUC and 70%–143% for C_{max}. What is the impact on bioequivalence studies? Int J Clin Pharmacol Ther 39: 350–355.

Herchuelz A (1996) Bioequivalence assessment and the conduct of bioequivalence trials: a European point of view. Eur J Drug Metab Pharmacokinet 21: 149–152.

Karim EF et al. (1988) An investigation of the metabolism of *S*-carboxymethyl-L-cysteine in man using a novel HPLC-ECD method. Eur J Drug Metab Pharmacokinet 13: 253–256.

Lutz D et al. (1985) The bioavailability and pharmacokinetics of two carbocysteine preparations after single and multiple dosing. Arzneim Forsch 35: 163–166.

Maynard WR, Bruce RB, Fox GG (1978) Determination of carbocysteine from human plasma. J Pharm Sci 67: 1753–1755.

Patnaik R et al. (1996) Bioequivalence assessment of generic drugs: an American point of view. Eur J Drug Metab Pharmacokinet 21: 159–164.

Staffeldt B, Brockmoller J, Roots I (1991) Determination of *S*-carboxymethyl-L-cysteine and some of its metabolites in urine and serum by high-performance liquid chromatography using fluorescent pre-column labelling. J Chromatogr 571: 133–147.