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Research Article

COMPARATIVE, OPEN LABEL, SINGLE DOSE CLINICAL TRIAL OF BIOAVAILABILITY OF FORMULATION CONTAINING AMBROXOL, TRIMETHOPRIM AND SULFAMETHOXAZOLE IN HEALTHY VOLUNTEERS

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ABSTRACT

Acute exacerbation of chronic bronchitis is a consequence of augmentation in air pathways secretions, often complicated by bacterial infections. Then, a clinical benefit can be anticipated with the join therapy of antimicrobial and mucolytic agents. In this study we aimed to compare the bioavailability and safety of an oral formulation containing ambroxol (AMBX), trimethoprim (TMP) and sulfamethoxazole (SMZ) in 24 healthy volunteers. Subjects were randomized to receive a tablet of (A) AMBX-TMP-SMZ (160, 800mg and 30mg); (B) TMP-SMZ (160mg and 800mg) and (C) AMBX (30mg), in a crossover way with 3 sequences in 3 periods (ABC, BCA, CAB) and 7 days of washout between each period. No significant changes were observed in the absorption indicators Cmax, Tmax, AUC0-t and AUC0-∞, and the elimination parameter T1/2 of SMZ, TMP or AMBX. Also Westlake 90% Confidence Intervals calculated for Cmax and AUC's were included in the bioequivalence range of 0.80-1.25 suggesting that the bioavailability of all agents in the new combined formulation is not different to that obtained following the individual administration of each. Volunteers claimed minimal side effects following all treatments. These results show the pharmacokinetic properties of a formulation containing TMP SMZ-AMBX that could contribute to improve the therapeutic adherence.

Keywords: Pharmacokinetic interaction, comparative bioavailability, ambroxol, cotrimoxazole.

INTRODUCTION

The World Health Organization (WHO) estimates that 1.9 million deaths occur every year from lower respiratory infections, primarily pneumonia, in developing countries. Because *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most common causes of childhood bacterial pneumonia in developing countries, WHO, using standardized case management guidelines, recommends using oral cotrimoxazole or amoxicillin to treat non-severe pneumonia at first-level health facilities[1,2].

On the other hand, clinical benefits from the use of mucolytic agents, such AMBX in the course of acute infections of the lower respiratory tract acute have been previously documented. In addition to this, AMBX has shown secretolytic, anti-inflammatory and local anesthetic activity[3].

Chronic obstructive pulmonary disease (COPD) is one of the main causes of morbidity and mortality worldwide. The prevalence of COPD in Mexico is high and factors such smoking, environmental pollution among other are risk factors worsening the disease course. Acute exacerbation of chronic bronchitis is a consequence of the augmentation in secretions of airways and inflammation commonly complicated with bacterial infections. Then a cycle of infection-inflammation-tissue injury-infection triggers a progressive obstruction of the airways[4]. In such cases not only antibiotic therapy is indicated but also mucolytic agents have been suggested to help to clear congestion in the chest by reducing sputum viscosity[5]. Moreover, some reports have proposed that the concomitant use of a mucolytic agent facilitates the penetration of antibiotic to pulmonary tissue and increases its concentration in bronchoalveolar lavage fluid[6].

Therefore the development of a combined therapy of an antibiotic and a mucolytic agent may appear suitable for increasing the pharmacologic efficacy and improving the patient compliance since only one medicine is needed[7]. In any case the evaluation of drug bioavailability in a combined formulation against that of individual components is required to prove a possible pharmacokinetic interaction. The present study was designed to evaluate the bioavailability and safety of AMBX, SMZ and TMP contained in a solid formulation containing AMBX 30 mg + SMZ 800 mg + TMP 160 mg as compared to that of the individual drugs following a single dose administration in a crossover 3-period design.

MATERIALS AND METHODS

Study Population

Twenty-four normal healthy mestizo volunteers (12 males and 12 females) aged from 19 to 34 years, weighing from 49.5 to 81.5 kg, measuring from 150 to 178 cm in height and from 20.45 to 30.0 Kg/m^2 in body mass index (BMI) were included. All the volunteers gave written informed consent after they had received detailed instructions about the aims, restrictions and possible adverse effect which could be experienced as a result of taking the drugs. The study was approved by the Ethics Committee of the Instituto de Investigación Clínica de Occidente S. A. de C.V. in Guadalajara, Jalisco, Mexico. Qualified medical staff made physical examinations, electrocardiogram (ECG) recording, hematological and urinary laboratory tests to all the volunteers to establish the health condition. The demographic characteristics of the study population are summarized in Table 1. Subjects did not take any other medications for at least 2 weeks prior to and throughout the entire study. Each subject was fasted overnight prior to the experiment, and food was withheld for 4 h after dosing. All medicines were administered with 250 mL of tap water following a 12 h fasting period. A standard low-fat lunch was given to all subjects 4 h after dosing.

Study Design

This was a single-center, open, randomized, single-dose, 3-period crossover study. The study was carried out as an open labeled trial, because changes in pharmacokinetic parameters were not expected as a result of this condition. Volunteers were randomized into three groups (A,B,C) to receive a single dose of A) a tablet containing AMBX-TMP-SMZ (160, 800 mg and 30 mg, respectively, Brogamax®, Farmacéuticos Rayere S.A.); B) a tablet containing TMP-SMZ (160 mg and 800 mg respectively, Bactrim F® Roche S.A. de C.V.); or C) a tablet containing AMBX (30 mg, Mucosolvan® BoehringerIngelheimPromeco, S.A. de C.V.). Subjects were given in a crossover fashion (3 sequences ABC, BCA, CAB) one of the 3 treatments in each of the 3 periods with a one week washout period. Blood samples were withdrawn previously to drug administration and 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 14, 24, 32 y 36 h after the drug intake.

The safety was evaluated by monitoring adverse events (AE's) during the three periods. Each subject was questioned on the study days for any symptoms of such events. The AE's were analyzed for establishing the relationship with the treatments.

Sample Treatment and Drug Analysis

Plasma from heparinized blood samples was obtained by centrifugation and kept at -70° C until drug analysis. Drug plasma levels were assessed by using adapted methods of high performance liquid chromatography coupled to UV detection in the case of SMZ and TMP[8], and electrochemical detection in the case of AMBX[9]. Prior to use any assay was validated by following national and international guidelines[10,11].

Data Analysis

To compare the rate and extent of absorption as well as the elimination properties of the study drugs, the following pharmacokinetic variables were calculated for each volunteer and product using the actual plasma sampling times: the area under the plasma concentration curves (AUC_{0-t}) were calculated with the linear trapezoidal

rule[12]. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained directly from the plasma-concentration data. The $AUC_{0-\infty}$ was calculated by dividing the last measured concentration by the elimination rate constant and adding the result to the AUC_{0-t}. The elimination rate constant was calculated by least-squares regression, using the decreasing concentration points of each curve. All pharmacokinetic parameter were obtained by using standard noncompartmental analysis implemented within WinNonLin 2.1 (Pharsight Co. Mountain View, CA). A crossover analysis of variance of the bioavailability indicators C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ was used to evaluate the effects due to sequence, subject, period and formulation. The Westlake 90% CI of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for the ratio of each drug into the new formulation (test) and the individual drug (reference) was determined to assess the bioequivalence between different products using the equivalence interval of 0.80 and 1.25. T_{max} and $T_{1/2}$ were analyzed by means the non-parametric Wilcoxon signed-rank test and a statistically significant difference was considered at $p<0.05$.

RESULTS

Treatment with either the individual drugs (AMBX or SMZ+TMP) or the combination (AMBX+SMZ+TMP) was reasonably well tolerated. Assessment of clinical biochemistry, vital signs, ECG and physical examination did not reveal major changes from screening to the end of the study. The most frequently reported AE was a mild pain sensation along the injection site (three occurrences out of six AE's), while three volunteers showed oral edema. All of these AE's were considered as probably related to the study treatments. In one subject a severe oral edema was noticed at the end of the first period and was dropped-out. However no significant loss of the statistical power was observed when bioequivalence analysis was carried out with the remaining 23 subjects.

Figures 1-3 depict the mean time-courses of the plasma levels of the AMBX, SMZ and TMP respectively when given individually as compared to the new combined formulation. After a single dose of medications, plasma concentrations reached a peak between 78.0-81.8 ng/mL for AMBX; 53.9-49.2 µg/mL for SMZ and 1.9-2.1 µg/mL for TMP, in the individual and combined formulations respectively. The time required to reach the maximal plasma concentration was 2.1 to 2.0 h for AMBX; 3.0 to 2.8 h for SMZ and 2.6 to 2.1 h for TMP, in the individual and combined formulations respectively.

Thereafter, plasma concentrations of AMBX declined with a mean $T_{1/2}$ of 11.7 vs 11.6 h, while SMZ did 8.6 *vs* 7.9 h and TMP did 15.3 vs 17.0 h for the individual versus the combined formulations. AUC_{0-t} and infinity-extrapolated AUC values for individual and combined AMBX, SMZ and TMP was on average not different. A summarized view of all relevant pharmacokinetic parameters is shown in Table 2. There was no period, sequence and formulation effect for different treatments.

The C/I (combined/individual) ratios for the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were all between 95 % and 108 % for all three drugs (AMBX, SMZ and TMP). Moreover, Westlake 90% CI for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of AMBX, SMZ and TMP were all included into the acceptance range of 80-125% (Table 2). Also, no significant differences were observed for the T_{max} and $T_{1/2}$ of the two treatments in the three drugs. The potency of the test in the case of SMZ and TMP was almost 1.0000 for the AUC's and greater than 0.9000 for C_{max}. However, a lower potency resulted in the statistical test for AMBX in all parameters. The lowest potency was for $AUC_{0-\infty}$(0.5754).

DISCUSSION

Respiratory infections still remain as common cause of death in developing countries. WHO recommends using oral cotrimoxazole or amoxicillin to treat non-severe pneumonia at first-level health facilities because the lower cost of these therapies[1]. Most frequent symptoms of respiratory diseases include throat soreness and cough, thus the use of a mucolytic agent is a common clinical practice. In this study we aimed to evaluate the bioavailability of AMBX, SMZ and TMP contained in a solid formulation containing AMBX 30 mg + SMZ 800 mg + TMP 160 mg as compared to that of the individual drugs following a single dose administration in a crossover 3-period design.

The rationale behind this new formulation is that previous reports have suggested that AMBX alone or as its active metabolite bromhexine, is able to increase the levels of distinct antibiotics at the infection site in the lower respiratory tract[3,6]. Indeed, a controlled clinical trial in children with acute infections of the lower respiratory tract was carried out to see whether or not treatment with AMBX could bring about faster and better results. One hundred twenty were all given antibiotics plus, at random, either AMBX (1.5-2.0 mg/kg body weight orally) or a placebo. The duration of the trial was ten days. All the patients in both groups were clinically

cured. In addition, remission of the cough, of the chest pathological signs, as well as the improvement of the lung radio graphical pictures were faster in children treated with AMBX than in those who received the antibiotic alone[13].

Assessment for a pharmacokinetic interaction in combined formulations is required to guaranteed that no loss in efficacy nor an increase in the frequency or severity of adverse effects are related to alterations in drug circulating levels. Indeed, it has been suggested that there exist a direct relationship between the plasma concentration of cotrimoxazole (SMZ+TMP) and its antibiotic effect. Nevertheless antibiotic plasma levels are not directly related to the frequency or intensity of adverse effects of the drug, thus the therapeutic range of the combination should be carefully revisited to avoid accumulation in multiple dose treatments or when using combined therapies[14].

In the present work the mean SMZ and TMP plasma level profile produced by the administration of the combination AMBX+SMZ+TMP did not differ from that following the administration of cotrimoxazole alone. The observed SMZ and TMP plasma levels are in agreement to those previously reported in a Mexican population[15]. When analyzed by bioequivalence statistics no changes in the C_{max}, T_{max} or AUC (to last point and extrapolated to infinity) were observed for both SMZ and TMP following the administration of the combined formulation as compared to the individual drug. Latest may suggest that the magnitude and the time to onset of the antibiotic effect of SMZ and TMP are to be not different in both formulations. Moreover, since no differences in the elimination pattern ($T_{1/2}$) were observed it could be supposed that the risk of adverse effects due to accumulation is not different for both formulations, cotrimoxazole alone and combined with AMBX.

In regard to AMBX similar results were observed; this is, no change in the pharmacokinetic parameters in the combined formulation compared to the individual AMBX tablet was found. These data indicate that the release, absorption and elimination of AMBX from the experimental formulation are not different to those of the drug when administered individually. The potencies obtained for all parameters are below to that specified by both national and international guidelines (0.8000)[10,11]. However AMBX plasma concentrations were similar to those previously observed[9,16]. Despite that this study was not designed to demonstrate that AMBX is able to improve the penetration of SMZ+TMP in the site of action during a respiratory infection, it could be supposed that the AMBX contained in the new combined formulation will show pharmacokinetic and therapeutic properties not different to those of the individual AMBX.

CONCLUSION

In conclusion, no significant changes were observed in the absorption indicators C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$, and the elimination parameter $T_{1/2}$ of AMBX, SMZ or TMP showing that the bioavailability of all agents in the new combined formulation is not different to that obtained following the individual administration of each agent. Although our study does not predict the clinical performance in patients, it is probably that the antibiotic and mucolytic response provided by the new formulation would be equivalent to that obtained with individual administration of TMP-SMZ and AMBX. Further studies should be performed to demonstrate the clinical efficacy.

COMPETING INTERESTS

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Table 1: Demographic characteristics of the study population (n = 24)

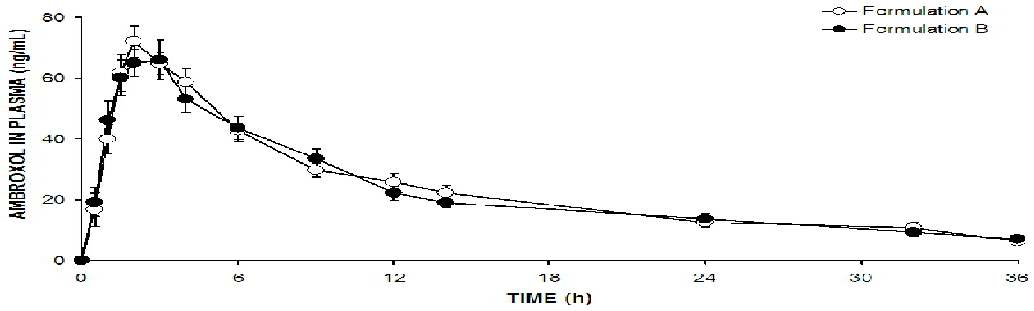
	Mean	SEM
Male volunteers (%)	50.00	N/A
Age (years)	23.67	0.89
Height (cm)	164.46	1.60
Weight (Kg)	66.70	1.73
BMI (Kg/m^2)	24.67	0.58

SEM = Standard error of the mean;
N/A = Not applicable;
BMI = Body mass index

Table 2: Pharmacokinetic parameters of AMBX, SMZ and TMP of the three evaluated formulations (mean ± SEM)

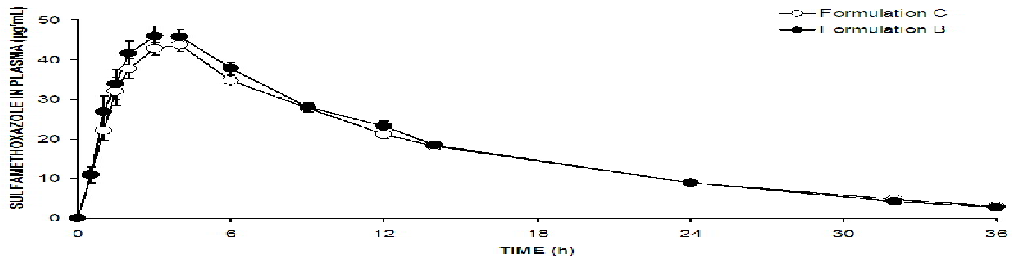
AMBROXOL					
Parameter	**Alone (A)**	**Combination(B)**	**Significance**	**Ratio%**	**Westlake 90 % CI**
C_{max} (ng/mL)	81.8 (5.1)	78.0 (6.4)	NS	95.5	85.4-114.6
T_{max} (h)	2.1 (0.2)	2.0 (0.1)	NS[a]		
AUC $_{0-t}$ (ng·h/mL)	917.3 (77.0)	909.3 (73.8)	NS	98.1	84.5-115.4
AUC $_{0-\infty}$ (ng·h/mL)	783.0 (64.9)	773.9 (69.4)	NS	99.4	84.9-115.1
$T_{1/2}$ (h)	11.7 (1.2)	11.6 (0.8)	NS[a]		
SULFAMETHOXAZOLE					
Parameter	**Alone (C)**	**Combination(B)**	**Significance**	**Ratio%**	**Westlake 90 % CI**
C_{max} (µg/mL)	49.2 (1.9)	53.9 (2.0)	NS	108.8	84.6-115.4
T_{max} (h)	3.0 (0.2)	2.8 (0.2)	NS[a]		
AUC $_{0-t}$ (µg·h/mL)	654.8 (17.3)	673.6 (21.3)	NS	103.2	92.5-107.4
AUC $_{0-\infty}$ (µg·h/mL)	614.4 (14.7)	640.3 (18.7)	NS	101.8	93.54-106.46
$T_{1/2}$ (h)	8.6 (0.4)	7.9 (0.4)	NS[a]		
TRIMETHOPRIM					
Parameter	**Alone (C)**	**Combination(B)**	**Significance**	**Ratio%**	**Westlake 90 % CI**
C_{max} (µg/mL)	2.1 (0.1)	1.9 (0.1)	NS	107.6	85.7-114.8
T_{max} (h)	2.6 (0.4)	2.1 (0.2)	NS[a]		
AUC $_{0-t}$ (µg·h/mL)	39.4 (2.7)	39.4 (2.3)	NS	101.6	93.9-106.1
AUC $_{0-\infty}$ (µg·h/mL)	30.4 (1.7)	29.3 (1.4)	NS	96.4	90.7-109.3
$T_{1/2}$ (h)	15.3 (1.5)	17.0 (1.1)	NS[a]		

C_{max}: peak plasma level; **T_{max}**: time to peak; **AUC_{0-last}** area under the concentration-time curve from time zero to last point of sampling; **$AUC_{0-\infty}$**: area under the concentration-time curve from time zero to infinity; **$T_{1/2}$**: terminal half-life. **Formulation A**: Ambroxol; **Formulation C**: Sulfamethoxazole+Trimethoprim; **Formulation B**: Ambroxol+Sulfamethoxazole+Trimethoprim.
[a]non significant by ANOVA.



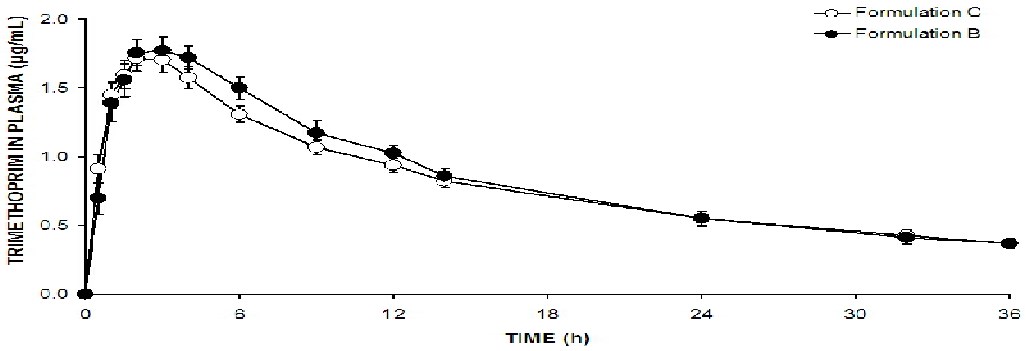
Mean (± SEM) time-course of ambroxol plasma levels in healthy volunteers in the comparative study of bioavailability of the drug when is given alone (formulation A, blank circles) or combined with cotrimoxazol (formulation B, SMZ-TMP-AMBX; full circles).

Fig. 1: Time-course of ambroxol plasma levels



Mean (± SEM) time-course of sulfamethoxazole plasma levels in healthy volunteers in the comparative study of bioavailability of the drug when is given alone (as SMZ-TMP (formulation C, blank circles) or combined with ambroxol (formulation B, SMZ-TMP-AMBX; full circles)

Fig. 2: Time-course of sulfamethoxazole plasma levels



Mean (± SEM) time-course of trimethoprim plasma levels in healthy volunteers in the comparative study of bioavailability of the drug when is given alone (formulation C, as SMZ-TMP; blank circles) or combined with ambroxol (Formulation B, SMZ-TMP-AMBX; full circles)

Fig. 3: Time-course of trimethoprim plasma levels

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