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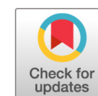

Assessment of Diet-Related Changes on Albendazole Absorption, Systemic Exposure, and Pattern of Urinary Excretion in Treated Human Volunteers

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ABSTRACT Soil-transmitted-helminth (STH) infections are a persistent global public health problem. Control strategies for STH have been based on the use of mass drug administration (MDA) mainly targeting preschool- and school-aged-children, although there is increasing interest in expanding treatment to include adults and others through community-wide MDA. Coverage assessment is critical to understanding the real effectiveness of albendazole (ALB) treatment in those MDA programs. The work described here aims to (i) evaluate the effect of type of diet (a heavy or light meal) and fasting before ALB treatment on the systemic disposition of ALB and its metabolites in treated human volunteers and (ii) evaluate the potential feasibility of detecting albendazole metabolites in urine. The data reported here demonstrate that the systemic availability of the active ALB-sulfoxide (ALBSO) metabolite was enhanced more than 2-fold after food ingestion (a heavy or light meal). ALB dissolution improvement related to the ingestion of food may modify the amount of drug/metabolites reaching the parasite, affecting drug efficacy and the overall success of MDA strategies. The measurement in urine samples of the amino-ALB-sulfone ($NHALBSO_2$) derivative and ALBSO for up to 96 h suggests that it may be feasible to develop a noninvasive tool to evaluate compliance/adherence to ALB treatment.

KEYWORDS albendazole, helminths, pharmacokinetics

Anthelmintic drugs are a key component for the control of the neglected tropical diseases (NTD) managed through mass drug administration (MDA) guided by the World Health Organization strategy of preventive chemotherapy, which involves periodic treatment of target populations based on baseline prevalence (1). The success of control programs is highly dependent on the proportion of the target population that takes treatment (coverage) and on the efficacy of the medications against each species of helminth involved (2, 3). Albendazole (ALB) is a critical drug for two of the NTDs with the highest burden, lymphatic filariasis and soil-transmitted helminthiasis (STH) (4). In the case of STH, there is a high variability in the efficacy of ALB, as well as mebendazole, against the different STH species (*Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*) which are currently targeted in control programs. Prior efficacy studies have demonstrated cure rates of >90% for *A. lumbricoides* but substantially lower efficacy against *T. trichiura* (5, 6).

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ALB is a highly effective benzimidazole derivative which has been demonstrated to be safe for the treatment of the common species of intestinal helminths seen in humans (7). Due to its low water solubility, ALB is formulated as tablets/suspensions and administered only by oral route in humans given as a 400 mg dose regardless of body weight. After oral treatment, drug particles must dissolve in the gastrointestinal (GI) fluids to facilitate absorption through the intestinal mucosa. Since the water solubility of benzimidazole compounds drastically increases at extreme pH values (8), the acidic pH of the stomach plays a central role in ALB dissolution. The undissolved drug particles pass down the GI tract and are excreted in feces without any chance to exert their anthelmintic action. Only the dissolved drug in solution is available for parasite acquisition at the GI tract, primarily by passive diffusion across the cuticle/tegument. Thus, the systemic exposure (plasma levels) of ALB and/or its active sulfoxide metabolite (ALBSO) reflects the amount of dissolved drug available at the GI level. Drug dissolution is a main limiting step in determining drug efficacy for benzimidazole anthelmintics (9).

Current methods for assessing coverage often rely on self-report through an interview-based coverage survey (10). The development of a tool to accurately determine coverage/adherence to MDA may allow more accurate and unbiased assessment of coverage and serve as an important monitoring and evaluation tool for programs.

Even when high levels of program coverage and compliance with ALB or mebendazole treatment are achieved and sustained, the success of programs in reducing transmission is also influenced by a wide range of other factors such as treatment frequency, age, seasonality, infection intensity, and selection of resistant parasites (10). Therefore, it is critical to differentiate a lack of adequate coverage or adherence to ALB from the development of anthelmintic resistance (5) and/or treatment failures related to the exposure of parasites to subtherapeutic drug concentrations that may occur due to poor drug dissolution, insufficient GI absorption, and/or systemic availability of the active ingredient (9).

The present work is a "second phase" of our previous study (11), focusing on the effect of different types of diets (light or heavy meal) or fasting on the systemic/urinary exposure of ALB/metabolites in ALB-treated humans. In addition, we assess the feasibility of detecting ALB metabolites in urine (including $NHALBSO_2$) as a possible tool to monitor adherence to MDA programs focused on STH control.

RESULTS

Subjects and carryover effect. All adult volunteers (12) completed the trial. No adverse events were recorded. No "phase effect" (carryover effect) was observed in serum or in urine. None of the main pharmacokinetic parameters in serum or urine showed statistical differences ($P > 0.05$) between phases.

Serum pharmacokinetics disposition of albendazole metabolites. ALBSO was the main analyte recovered in serum between 2 and 72 h posttreatment (p.t.). Low $ALBSO_2$ concentrations were quantified between 2 and 36 h p.t. Only trace amounts of ALB were detected between 2 and 12 h p.t. and only in some volunteers. Most $ALBSO_2$ serum concentrations were under the limit of quantification (LOQ) which precluded any pharmacokinetic analysis. The serum pharmacokinetic analysis was carried out only for ALBSO.

The comparative (mean ± standard deviation [SD]) serum concentration profiles of ALBSO, $ALBSO_2$, and ALB obtained after the oral administration of ALB (400 mg) and the concentrations (mean ± SD) of ALBSO in samples coming from female and male are shown in Fig. 1. Marked differences in the serum concentration profile of ALBSO were observed among fasted volunteers (group III) and those receiving either a heavy meal or a light meal (groups I and II, respectively). There were no clinically meaningful differences observed by gender in the main pharmacokinetics parameters of ALBSO.

The correlation between either age or body weight and ALBSO systemic exposure was not statistically different ($P > 0.05$). Fig. 2 shows the comparative serum concentration profiles (mean ± SD) of ALBSO observed in human volunteers treated with ALB belonging to groups I, II, and III. Food ingestion before ALB treatment affected the serum disposition kinetics of ALBSO in treated humans. The most important differences were

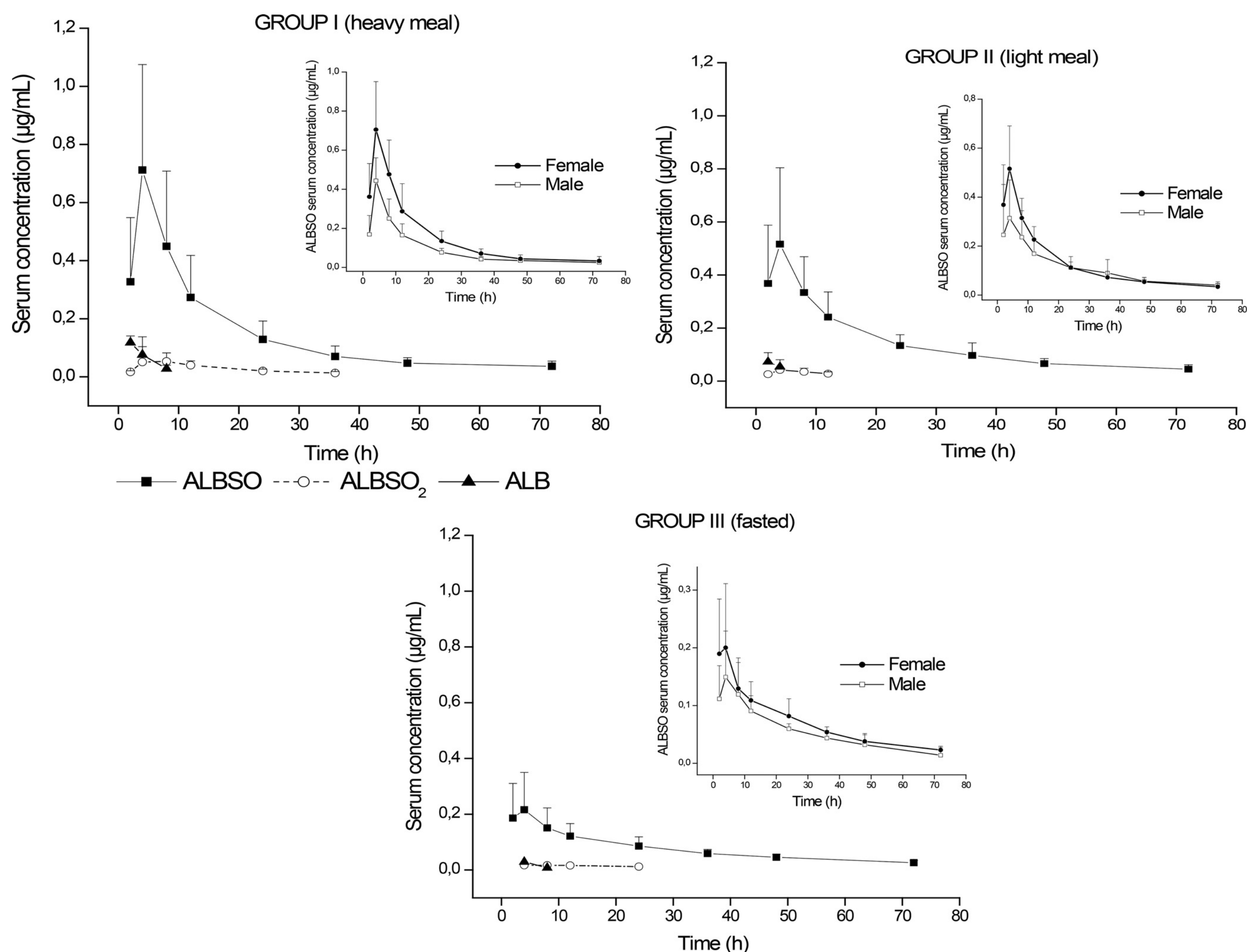

FIG 1 Comparative (mean ± SD) serum concentration profiles of albendazole sulfoxide (ALBSO), albendazole sulfone ($ALBSO_2$), and albendazole (ALB), obtained after oral administration of ALB (400 mg) to human volunteers either fasted during 8 h (group III) or receiving a heavy (group I) or light (group II) meal. The inset shows the comparative (mean ± SD) serum concentration profiles of ALBSO according to the gender in each group.

found between 2 and 24 h p.t. After that, the concentrations decreased rapidly in all groups. ALBSO was detected until 72 h over the LOQ (0.025 μg/ml) in just 5 volunteers from group III, instead of 11 from groups I and II. The serum peak concentration (C_{max}) was achieved 4 h after ALB administration, in either the fed or the fasted volunteers. The C_{max} observed in group III (0.23 ± 0.14 μg/ml) was significantly lower ($P < 0.05$) than that observed in group I (0.71 ± 0.36 μg/ml) and group II (0.52 ± 0. 29 μg/ml). Similar differences in ALBSO systemic exposure (measured as AUC_{0-LOQ}) were observed. Table 1 summarizes the main serum pharmacokinetic parameters obtained for ALBSO in human volunteers treated with ALB. Diet did not affect ALBSO depletion, since the elimination half-lives were statistically similar ($P > 0.05$) among volunteers who received a high-fat meal (16.4 ± 4.75 h), received a light meal (22.9 ± 5.88 h), or were fasted during 8 h before ALB administration (25.1 ± 10.6 h). ALBSO formation half-life ($t_{1/2\ for}$) and mean residence time (MRT) did not show differences among groups.

Urinary excretion of albendazole metabolites. ALBSO and $NHALBSO_2$ were the only analytes detected and suitable to undergo a pharmacokinetic analysis in volunteers treated with ALB. $ALBSO_2$ could be measured in some samples, but the majority of these measurements were below the LOQ.

ALBSO and $NHALBSO_2$ were rapidly excreted in urine and quantified from the first sampling time (2 h) up to 96 h p.t. As shown in Fig. 3, the diet/fast condition before

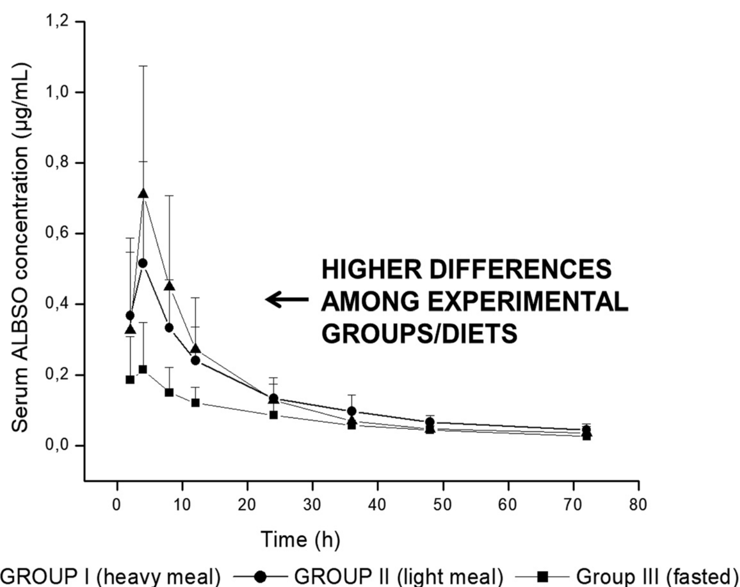

FIG 2 Comparative (mean ± SD) serum concentration profiles of albendazole sulfoxide (ALBSO) obtained after albendazole administration (400 mg) to human volunteers either fasted (group III) or receiving a heavy (group I) or light (group II) meal.

ALB administration modified urinary excretion profile of ALB metabolites. The concentrations of both metabolites resulted in group I > group II > group III. In all experimental groups, ALBSO concentrations in urine were higher than those observed for $NHALBSO_2$, mainly in the period of 2 up to 24 h p.t., and the peak concentration (C_{max}) was significantly higher ($P < 0.05$). However, the difference in urinary excretion between both metabolites expressed as AUC_{0-LOQ} was significantly higher only in group I (Fig. 4A). The detection period of both metabolites in urine under our experimental conditions was similar among groups. They were detected over the LOQ (0.01 μg/ml) in 100% of urine samples taken until 48 h (ALBSO) or from 4 to 48 h ($NHALBSO_2$). Both analytes were quantified in 91% of samples taken up to 72 h post-ALB administration and in more than 50% of samples taken at 84 and 96 h p.t. The urine excretion profile of each metabolite is shown in Fig. 4B. Diet-induced changes in ALBSO urine concentrations were observed mainly between 2 to 24 h. Between 36 to 96 h p.t., ALBSO concentrations in each sampling point were similar among groups ($P > 0.05$). ALBSO achieved the highest concentrations in urine after a heavy meal

TABLE 1 Pharmacokinetic parameters (mean ± SD) of albendazole sulfoxide (ALBSO) obtained in serum after albendazole (ALB) administration as a single oral dose (400 mg) to human volunteers under different dietary regimens[a]

Pharmacokinetic parameters[b]	Group I (heavy meal)	Group II (light meal)	Group III (fasted)
Cmax (μg/ml)	0.71 ± 0.36 A	0.52 ± 0.29 A	0.23 ± 0.14 B
AUC_{0-LOQ} (μg·h/ml)	10.4 ± 4.77 A	9.76 ± 3.42 A	5.06 ± 1.73 B
$AUC_{0-\infty}$ (μg·h/ml)	11.7 ± 4.56 A	11.7 ± 3.6 A	5.77 ± 2.15 B
t_{max} (h)	4.00 ± 0.00 A	3.83 ± 1.58 A	3.83 ± 1.58 A
$t_{1/2\ el}$ (h)	16.4 ± 4.75 A	22.9 ± 5.88 A	25.1 ± 10.6 A
$t_{1/2\ for}$ (h)	1.93 ± 1.86 A	1.66 ± 0.73 A	1.99 ± 2.88 A
MRT (h)	24.9 ± 8.33 A	30.7 ± 8.06 A	28.4 ± 12.2 A
Normalized C_{max}	0.57 ± 0.23 A	0.42 ± 0.20 A	0.18 ± 0.09 B
Normalized AUC_{0-LOQ}	8.52 ± 3.49 A	8.13 ± 2.71 A	4.18 ± 1.09 B

[a]Different letters (A, B) correspond to statistically significant differences between pharmacokinetic parameters ($P <$ 0.05).

[b]C_{max}, peak serum concentration; AUC_{0-LOQ}, area under the concentration versus time curve from time zero to the limit of quantification; $AUC_{0-\infty}$, area under the concentration versus time curve extrapolated to infinity; $t_{1/2\ for}$, formation half-life; t_{max}, time to peak serum concentration; $t_{1/2\ el}$, elimination half-life; MRT, mean residence time. Normalized C_{max} and normalized AUC_{0-LOQ}: values normalized according to the lower dose (5.48 mg/kg).

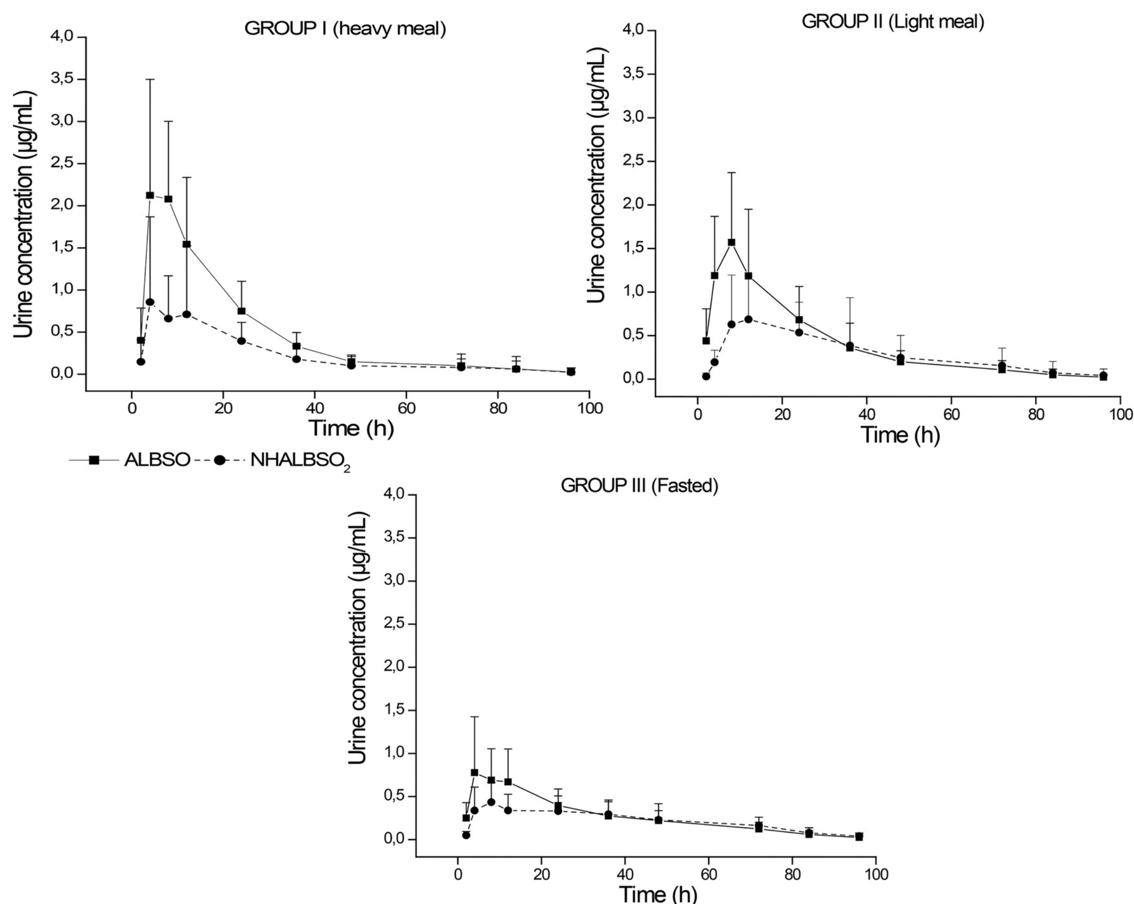

FIG 3 Comparative (mean ± SD) urine concentration profiles of albendazole sulfoxide (ALBSO) and amino albendazole sulfone ($NHALBSO_2$) obtained after oral administration of albendazole (400 mg) to human volunteers either fasted (group III) or receiving a heavy (group I) or light (group II) meal.

(group I) and the lowest one after 8 h of fasting (group III). Between 7 and 10 h p.t., ALBSO reached its peak concentration (C_{max}) in urine, which is significantly higher ($P < 0.05$) in group I (2.66 ± 1.23 μg/ml) than in group II (1.72 ± 0.75 μg/ml) and group III (0.93 ± 0.58 μg/ml). The ALBSO urinary excretion (expressed as AUC_{0-LOQ}) was similar in groups I and II (fed groups) and significantly lower ($P < 0.05$) in group III (fasted).

Differences in $NHALBSO_2$ urine concentrations among groups were not as pronounced. The $NHALBSO_2$ urine excretion C_{max} observed in groups I and II ($P < 0.05$) was higher than that observed in group III. However, these differences were not reflected in significant differences in the AUC_{0-LOQ} values among groups. Table 2 summarizes the main pharmacokinetic parameters obtained in urine for ALBSO and $NHALBSO_2$ in human volunteers subjected to different diets/fasting before ALB administration.

Correlation between serum disposition and urinary excretion of albendazole metabolites. Fig. 5 compared ALB metabolites concentrations in serum and in urine after ALB administration. In the three experimental groups, ALBSO and $NHALBSO_2$ were quantified in urine at a higher concentration than that observed for ALBSO in serum. The ALBSO C_{max} in urine was delayed from 4 (T_{max} in serum) to 8 h p.t., and it was approximately 4-fold higher than that observed in serum. Similar results were observed for the AUC_{0-LOQ} value since it was more than 4-fold higher in urine than in serum (Fig. 5). A positive correlation was observed between the ALBSO concentration in serum and in urine with a Pearson coefficient value (r) of 0.841, 0.682, and 0.716 for groups I, II, and III, respectively, considering all sampling points (2 to 72 h). However, the

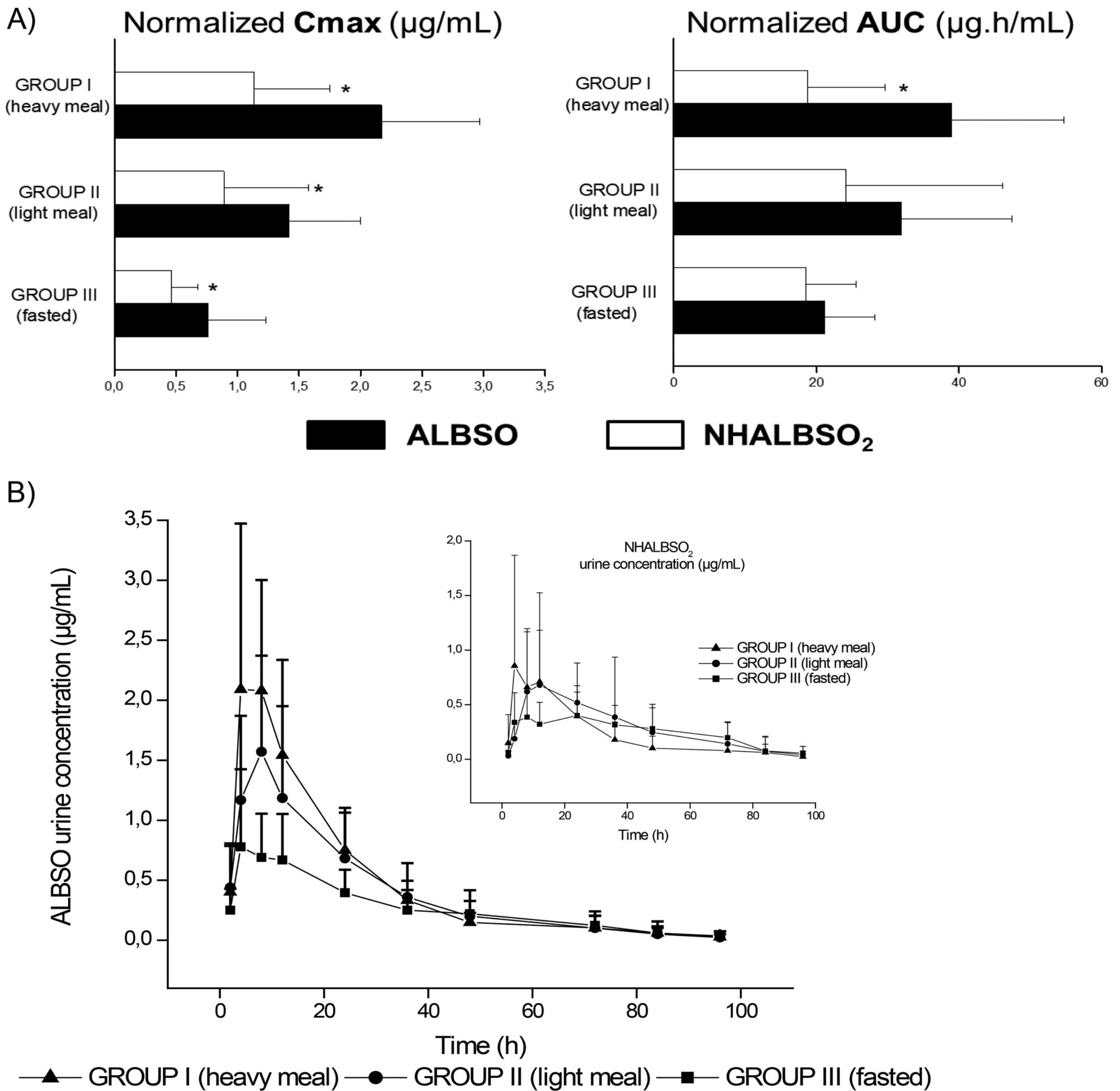

FIG 4 (A) Differences in the mean (± SD) of the peak concentration (C_{max}) and area under the concentration versus time curve (AUC_{0-LOQ}) between albendazole sulfoxide (ALBSO) and amino albendazole sulfone ($NHALBSO_2$) metabolites for each group. *, statistically significant differences ($P < 0.05$). (B) Comparative (mean ± SD) urine concentration profiles of ALBSO among groups. The inset shows the comparative (mean ± SD) urine concentration profiles of $NHALBSO_2$ among them.

correlation was more accurate at sampling points between 8 and 72 h p.t., with *r* values of 0.988 (group I), 0.996 (group II), and 0.984 (group III).

DISCUSSION

The dissolution and absorption of ALB may be modified significantly by diet, resulting in substantial variation in the amount of active drug metabolites reaching the parasite. This variability may have important implications for drug efficacy and ultimately may affect overall success of STH control programs. ALB treatment in humans does not always result in the expected therapeutic success. The efficacy of a single ALB dose,

TABLE 2 Pharmacokinetic parameters (mean ± SD) for albendazole sulfoxide (ALBSO) and amino albendazole sulfone ($NHALBSO_2$) obtained in urine after albendazole (ALB) administration as a single oral dose (400 mg) to human volunteers undergoing different diet regimens[a]

	Group I (heavy meal)		Group II (light meal)		Group III (fasted)	
Pharmacokinetic parameters[b]	ALBSO	$NHALBSO_2$	ALBSO	$NHALBSO_2$	ALBSO	$NHALBSO_2$
C_{max} (μg/ml)	2.66 ± 1.23 A	1.41 ± 0.94 A	1.72 ± 0.75 B	1.06 ± 0.88 A	0.93 ± 0.58 C	0.54 ± 0.25 B
$AUC_{0–LOQ}$ (μg·h/ml)	46.1 ± 17.9 A	22.2 ± 12.4 A	38.6 ± 20.4 A	28.4 ± 23.3 A	25.7 ± 10.8 B	21.9 ± 7.69 A
$AUC_{0–\infty}$ (μg·h/ml)	46.8 ± 18.8 A	23.4 ± 14.5 A	43.3 ± 26.1 A	31.9 ± 24.0 A	26.9 ± 11.8 B	23.6 ± 8.65 A
t_{max} (h)	7.33 ± 3.33 A	8.67 ± 5.86 A	7.67 ± 2.67 A	16.3 ± 8.70 A	10.1 ± 9.03 A	17.7 ± 14.6 A
$t_{1/2\ el}$ (h)	13.4 ± 5.26 A	17.3 ± 12.5 A	13.9 ± 4.62 A	18.9 ± 15.1 A	22.4 ± 10.9 A	26.6 ± 9.11 A
$t_{1/2\ for}$ (h)	2.83 ± 2.29 A	5.24 ± 3.61 A	3.23 ± 1.66 A	5.86 ± 3.81 A	2.90 ± 3.16 A	4.74 ± 4.90 A
MRT (h)	21.1 ± 6.55 A	26.5 ± 17.4 A	22.8 ± 5.37 A	27.3 ± 10.7 A	33.5 ± 12.8 B	43.3 ± 12.7 B
Normalized C_{max}	2.17 ± 0.79 A	1.13 ± 0.61 A	1.42 ± 0.58 B	0.88 ± 0.68 A	0.70 ± 0.47 C	0.46 ± 0.21 B
Normalized $AUC_{0–LOQ}$	38.9 ± 15.7 A	18.7 ± 10.8 A	31.8 ± 15.5 A	24.2 ± 21.8 A	21.1 ± 7.10 B	18.5 ± 7.00 A

[a]Different letters (A, B, C) indicates statistically significant differences among groups for either ALBSO or $NHALBSO_2$ ($P < 0.05$).
[b]C_{max}, peak urine concentration; $AUC_{0–LOQ}$, area under the concentration versus time curve from time zero to the limit of quantification; $AUC_{0–\infty}$, area under the concentration versus time curve extrapolated to infinity; $t_{1/2\ for}$, formation half-life; t_{max}, time to peak urine concentration; $t_{1/2\ el}$, elimination half-life; MRT, mean residence time. Normalized C_{max} and normalized $AUC_{0–LOQ}$, values normalized according the lowest standard dose (5.48 mg/kg).

although highly efficacious against *A. lumbricoides* and hookworms, has poor performance against *T. trichiura*. Cure rates below 30% and as low as 2.6% have been reported (5). Understanding the pharmacokinetic behavior of ALB in humans and the main factors that can modify this behavior will contribute to increasing efficacy against STH "refractory" to chemical control. The aim of this study was to determine the relationship between the type of diet and the systemic exposure of ALB/metabolites in human volunteers. Our findings confirm that the parent compound is almost undetectable in the serum after ALB administration, which is attributed to first-pass oxidation in the liver (13). Both the ALB metabolites profile and the high variability in serum exposure observed among volunteers are in agreement with previous studies performed in humans (14–17).

The systemic availability of the active ALBSO metabolite was influenced by food intake, mainly during the first 24 h after ALB administration. Statistical differences were found in ALBSO C_{max} and $AUC_{0–LOQ}$ values between fed and fasting conditions. These pharmacokinetic parameters were enhanced more than 2-fold in serum when ALB was administered after 30 min of either a fatty or a light meal. Similar results have also been reported in other human studies, where an increase in the ALB absorption and resultant ALBSO concentration in plasma was observed with food intake before administration (18–21). Changes in the GI absorption of ALB modulated by diet have been demonstrated in different animal species (22–25). In ruminants, starvation decreases the flow rate of the digesta through the GI tract, improving drug dissolution, absorption, and exposure of benzimidazole compounds (9, 24, 26).

In our study, the type of diet ingested before ALB administration (fatty or light meal) did not significantly affect the serum disposition kinetics of ALBSO. This would indicate that a minimum intake content would be enough to decrease stomach pH favoring drug dissolution/absorption. The lack of differences could also be explained by the high interindividual variability attributed to differences in gastric pH and metabolism, as well as poor and/or erratic absorption of ALB, among other host-related factors (17, 19, 27).

ALB shows limited GI absorption due to its poor water solubility, which has been shown to increase markedly at low pH values (27). Thus, the time of residence of the compound (tablet) at the acidic environment of the stomach affects the amount of dissolved active principle. Only the dissolved drug is available for absorption and diffusion through the external surface of parasites located at the GI lumen. Consequently, the higher the drug quantified in the bloodstream (higher systemic exposure), the greater the amount of drug available at the GI level, which accounts for enhanced anthelmintic activity. The findings reported here suggest that food may delay gastric emptying rate, increasing the dissolution of drug particles with consequent increase in the systemic availability of ALB/metabolites. ALB was administered in a fixed dose (400 mg) to

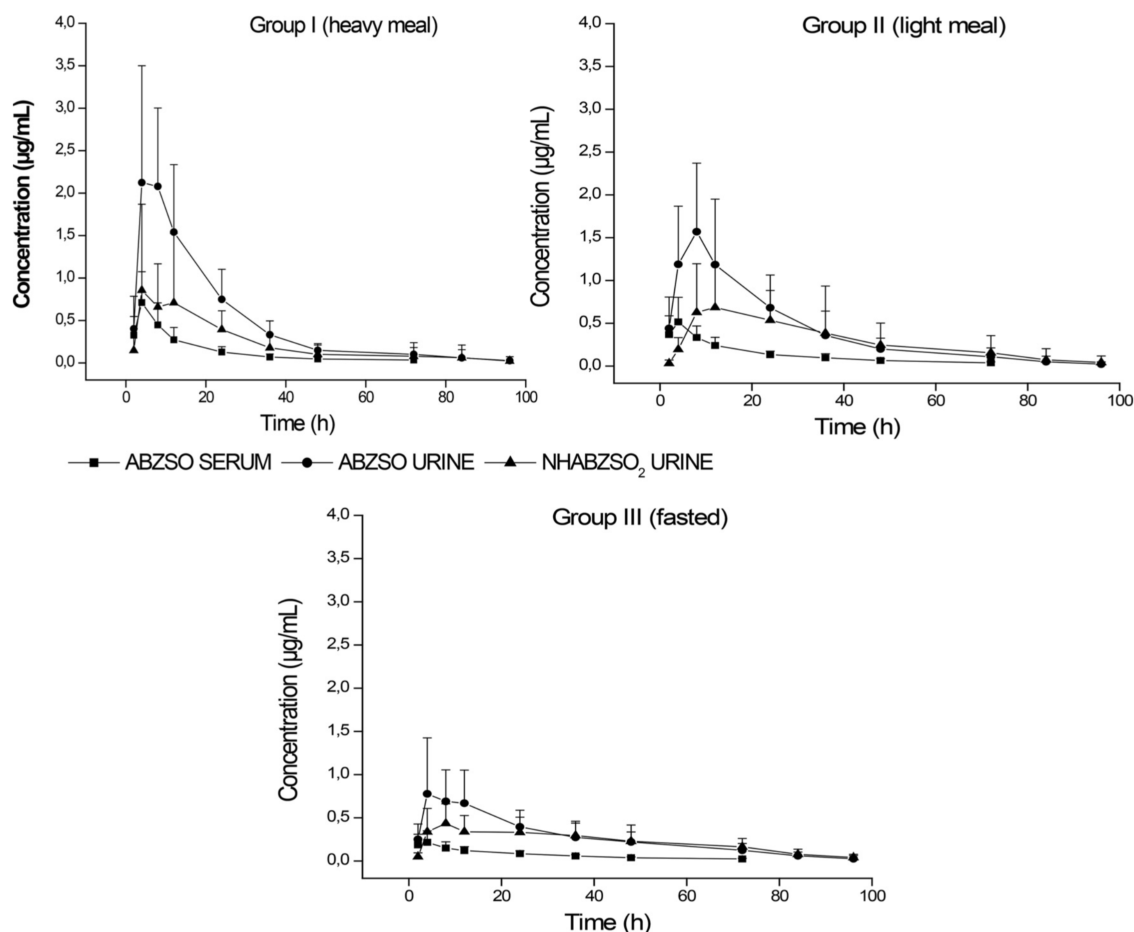

FIG 5 Comparative (mean ± SD) serum and urine concentration profiles of albendazole sulfoxide (ALBSO) and amino albendazole sulfone ($NHALBSO_2$) obtained from human volunteers, either fasted (group III) or receiving a heavy (group I) or light (group II) meal before oral administration of ALB (400 mg).

individuals with a body weight ranging from 46 to 76 kg, resulting in standard doses between 5.5 and 8.7 mg/kg. A positive correlation was observed between dose (mg/kg) and ALBSO systemic exposure. However, this only reached statistical significance for C_{max} in group III (fasting, $r = 0.61$, $P = 0.035$) and AUC in group I (heavy meal, $r = 0.71$, $P = 0.009$), which indicates that differences in ALBSO systemic exposure cannot be explained just by differences in the administered dose. Additionally, factors other than drug dissolution/availability may also account for observed differences in drug efficacy.

The anthelmintic efficacy of benzimidazole compounds, including ALB, depends on their ability to reach high and sustained concentrations at the site of parasite location and bind to the parasite β-tubulin (9). Drug accumulation depends on, among other variables, the concentration gradient and time of absorption. Studies done in domestic animals indicate that the time of drug exposure and drug concentration are important factors determining the efficacy of benzimidazole anthelmintics (9). A similar situation appears to occur in humans. In a recent study (6), a significantly higher cure rate and a significantly higher egg reduction rate were observed in *T. trichiura*-infected children after ALB oral treatment (400 mg) during 3 consecutive days compared to those after ALB single-dose. In the current work, ALBSO MRT values and time of drug exposure were similar among experimental groups. However, considering a similar time period (24 h), a higher systemic exposure for ALBSO was observed in those individuals who received food before ALB administration, which indicates a greater dissolution of the drug in the GI tract (mainly in the stomach). Intestinal nematodes are exposed to drug

dissolved in the gut fluids and also to drug recycled from plasma to the gut lumen (28). ALB systemic exposure correlates with drug levels measured in different tissues/fluids and within target parasites (29–31). Increased serum availability of active drug/metabolites accounts for enhanced clinical efficacy, and the characterization of the serum disposition kinetics of these molecules can be used to predict and optimize anthelmintic efficacy. These findings suggest that administering ALB with food can improve dissolution and absorption, which may ultimately improve drug efficacy.

A further experimental goal of the current study was to search for a reliable marker to monitor adherence/compliance of individuals to ALB treatment. The use of noninvasive urine sampling is painless, relatively convenient, and stable, and it may be more acceptable than blood sampling. In some instances, urine-based assays could provide coverage of MDA campaigns that is more reliable than that of traditional and subjective reporting methods currently used (10). We report here the urinary excretion profile of ALB/metabolites orally administered after food or fasting for 8 h. For the first time, we describe the pattern of the metabolite $NHALBSO_2$ in urine. This may represent a novel metabolite that can also be measured by the validated methodology to monitor treatment adherence. Analysis of urine for 96 h revealed the presence of ALBSO as the main metabolite, followed by the amino-derivative, in volunteers in either fed or fasting conditions. This metabolite has been identified in low concentrations in the bloodstream of pigs, humans, and cattle (20, 22, 32).

The metabolites ALBSO and $NHALBSO_2$ were detected over the LOQ in most urine samples of volunteers with or without food ingestion before ALB treatment. For ALBSO, urine exposure, expressed as AUC_{0-LOQ}, was 4-fold higher than that observed in serum, in either fed or fasting conditions, which is in agreement with our previous results (11). Although both ALB metabolites were detected in urine in all experimental groups, ALBSO concentrations were higher than those observed for $NHALBSO_2$, mainly in the period from 2 to 24 h p.t. In addition, ALBSO was detected above the LOQ (0.01 μg/ml) in 100% of the urine samples taken up to 48 h. Diet did not appear to induce significant changes in the $NHALBSO_2$ urine availability (expressed as AUC_{0-LOQ}). Both ALBSO and $NHALBSO_2$ were quantified in all urine samples up to 48 h posttreatment (groups I, II, and III) and, in most of them, until 72 h p.t. Thus, urine sampling within 2 days p.t. allows to determine accurately ALB metabolites concentrations and can be a useful tool to assess compliance/treatment coverage in MDA campaigns. Therefore, ALBSO is the metabolite of choice to look for in urine as an indicator of adherence to ALB treatment up to 24 h posttreatment. The measurement of either ALBSO or the $NHALBSO_2$ metabolite in urine samples can be used indistinctly as proof of treatment adherence after 24 and up to 48 h posttreatment. Additionally, the sampling period could be extended up to 96 h p.t. without a significant loss of accuracy, since both ALBSO or $NHALBSO_2$ were quantified above the LOQ in most of the volunteers.

In summary, our findings demonstrate that diet plays an important role in mediating the absorption of ALB and could be an important interventional tool to improve nematocidal efficacy and program effectiveness. In addition, the measurement of the $NHALBSO_2$ derivative in urine for 96 h p.t., moreover ALBSO metabolite, suggests that the development of a tool to objectively evaluate compliance/adherence to ALB treatment using a noninvasive sample may be feasible.

MATERIALS AND METHODS

Ethical aspects. The study protocol and informed consent form (ICF) was approved by the regulatory authorities of the Province of Salta, Argentina, Comité de Ética en Investigación, Comisión Provincial de Investigación en Ciencias de la Salud – COPICSa (EXP. 321-93864/2019). All volunteers provided written consent for their participation.

Experimental design: treatment and sampling. This trial was conducted at the facilities of the Instituto de Investigaciones de Enfermedades Tropicales, Universidad Nacional de Salta, Oran, Argentina, guided by the local regulations and following good clinical practices (GCP) and good laboratory practices (GLP). Twelve healthy adult volunteers (six female and six male, body weight between 46 and 73 kg, age between 22 and 36 years) participated in a crossover design with three different experimental

phases. In phase I, volunteers were randomly assigned to 1 of 3 experimental groups (n = 4 each, two females and two males). Group I (heavy meal): volunteers received a high-fat meal (estimated fat content 40 g) 30 min before ALB administration. Group II (light meal): volunteers received a light meal (estimated fat content 15 g) 30 min before ALB administration. Group III (fasted): volunteers were maintained for an 8-h fasting period before ALB administration.

Volunteers were crossed over between the other 2 groups, with a 14-day washout period between phases. All volunteers received 400 mg of ALB (tablet, GlaxoSmithKline, UK). The sample size calculation for this trial was based on the area under the concentrations versus time curve (AUC) data from a previous trial on serum and urine ALB kinetics in human volunteers (11). Twelve individuals provided >80% power to detect a 20% between-treatments difference with a 95% confidence interval.

Before ALB treatment, baseline blood (5 ml) and urine (10 ml) samples were obtained in each phase. Venous blood and urine samples were collected between 2 and 72 h or 2 and 96 h post-ALB treatment (p.t.), respectively. The samples were stored at −20°C until high-performance liquid chromatography (HPLC) analysis of ALB and its metabolites.

Pharmacokinetic studies: analytical phase. Serum sample cleanup/extraction. The methodology used in the current work to extract ALB and its metabolites, ALBSO and ALB-sulfone ($ALBSO_2$), from serum samples has been previously published (11).

Urine sample extraction. The urine extraction methodology of ALB/metabolites was adapted from that previously reported (11). Briefly, urine samples (5 ml) were placed into plastic tubes (10 ml) spiked with 60 μl of a solution (25 μg/ml) of oxibendazole (OBZ) as internal standard (IS) and the molecules to be assayed (ALB, ALBSO, $ALBSO_2$, and amine albendazole sulfone [$NHALBSO_2$]) in the validation procedure. The analytes were extracted by the addition of 4 ml of ethyl acetate. After shaking (vortex 80 min) and centrifugation (2500 × g, 15 min, 4°C), the clear supernatant (ethyl acetate phase) was transferred to a 10-ml glass tube, and the procedure was repeated. The total supernatants were evaporated and the dry residue was dissolved in 250 μl of mobile phase (acetonitrile:water, 27:73) and shaken (15 min).

HPLC system and chromatographic conditions. Experimental and fortified serum and urine samples were analyzed for ALB, ALBSO, $ALBSO_2$, and $NHALBSO_2$ by HPLC. After extraction, 50 μl of the sample was injected in a Shimadzu Chromatography System (Shimadzu Corporation, Kyoto, Japan). The equipment characteristics and chromatographic method were the same as those mentioned by Ceballos et al. (11).

Validation procedure. Full validation of the analytical procedures for the extraction and quantification of each molecule (ALB, ALBSO, $ALBSO_2$, and $NHALBSO_2$) in each biological matrix (serum and urine) was performed before the analysis of the experimental samples, following internationally recognized criteria (12).

The chromatographic identification of ALB and its metabolites was undertaken by comparison with retention times of pure (>99%) reference standards (Toronto Research Chemicals, Toronto, Canada). The blank serum and urine samples were free of interferences in the period of analytical interest. The linearity of the method was tested after the elaboration of analytical calibration curves which showed good linearity in both matrixes with correlation coefficients of ≥0.998. The calibration ranges for ALB and its metabolites were between 0.025 and 2 μg/ml in serum and between 0.01 and 5 μg/ml in urine. Mean absolute recovery percentage for ALB and its metabolites was ≥75.4% in serum samples and ≥70.7% in urine samples.

The limit of detection (LOD) for ALB/metabolites in serum was 0.001 and in urine was ≤0.009 μg/ml. The limit of quantification (LOQ), defined as the lowest measured concentration with a coefficient of variation (CV) of <20%, accuracy of ±20%, and absolute recovery of >70%, was 0.025 μg/ml in serum and 0.01 μg/ml in urine for ALB/metabolites. Concentration values below the LOQ were not considered for the pharmacokinetic analysis.

The stability of analytes was evaluated on blank serum/urine samples fortified with ALB, ALBSO, $ALBSO_2$, and $NHALBSO_2$ at two different concentrations (n = 5) and stored at −18°C. Samples were assayed after a 3-day period. Additionally, freeze/thaw studies were conducted (3 freezes/thaw cycles, 1 cycle per day) at two different concentrations (n = 5), and the stability to "tropical" temperature (32°C) was tested during 12 and 24 h of incubation (Versatile Environmental Test Chamber; SANYO Electric Co., Japan). Stability in human serum/urine was considered acceptable, since the coefficients of variation were always lower than 15% for ALB and its metabolites, indicating no significant degradation of molecules under these conditions.

Pharmacokinetic analysis. The pharmacokinetic analysis of the serum and urine concentrations obtained after ALB single oral administration (400 mg tablet) to human volunteers was carried out using the program PK Solution (Summit Research Services, Ashland, USA). The pharmacokinetic parameters were calculated in the same way as those reported by Ceballos et al. (11).

Statistical analysis of the data. The pharmacokinetic parameters and concentration data are reported as arithmetic mean ± SD. Parametric paired tests (analysis of variance [ANOVA] plus Tukey) were used for the statistical comparison of pharmacokinetic parameters obtained for the different experimental groups and evaluation of "phase effect." Differences in normalized C_{max} and AUC_{0-LOQ} values observed between male and female individuals in each experimental group were compared by Student's t test. Normalized C_{max} and AUC_{0-LOQ} values were calculated for each volunteer (V) as follows:

$$\text{Normalized } C_{max} = \frac{C_{max}\ \text{V} \times 5.48}{\text{V dose}}$$

$$\text{Normalized AUC} = \frac{\text{AUC V} \times 5.48}{\text{V dose}}$$

where C_{max} V or AUC V are the values of these parameters calculated for each V, 5.48 is the lowest administered standard dose (mg/kg) among volunteers, and V dose is the standard dose (mg/kg) received for each V. In all cases, a P value of <0.05 was considered statistically significant. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, La Jolla, CA).

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