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Article

Pharmacokinetics of isoniazid in Wistar rats exposed to ethanol

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Tuberculosis treatment consists of a drug combination, where isoniazid is the core drug and alcoholism is a factor highly related to poor patient compliance with the therapy. CYP2E1 is an enzyme involved both in the metabolism of ethanol and in the formation of hepatotoxic compounds during the metabolism of isoniazid. The shared metabolism pathway accounts for the possibility of pharmacokinetic interaction in cases of concomitant alcohol use during tuberculosis treatment. The aim of this study was to evaluate the effect of repeated exposure of Wistar rats (males, 250 g, n=6) to ethanol on the pharmacokinetics of a single dose of isoniazid in combination with pyrazinamide and rifampicin (100 mg/kg, 350 mg/kg and 100 mg/kg, respectively). An animal group received the combination of drugs and ethanol and was compared to a control group, which received the combination of drugs without exposure to ethanol. The plasma concentrations of isoniazid were determined by a UHPLC/UV bioanalytical method that was previously validated. Biochemical markers of liver function were measured to assess potential damage. A lower elimination half-life of isoniazid was observed in the ethanol group than in the control group (t1/2 0.91 h versus 1.34 h). There was no evidence of hepatotoxicity through the biomarker enzymes evaluated. The results allow us to infer that although there are no biochemical changes related to liver damage, there is a slight influence of ethanol exposure on the pharmacokinetic profile of isoniazid. This change may have a relevant impact on the efficacy of isoniazid in the outcome of tuberculosis treatment.

Keywords: Isoniazid. Ethanol. Pharmacokinetic interaction. Bioanalytical Method. Tuberculosis.

INTRODUCTION

Tuberculosis is a contagious infectious disease mainly caused by *Mycobacterium tuberculosis* and is a public health concern worldwide. Approximately one-third of the world's population is infected. In 2017 alone, there were 10 million new cases and 1.3 million deaths due to the disease (WHO, 2018).

The recommended dose regimen for its treatment is the use of isoniazid (INH), rifampicin (RMP), pyrazinamide (PYR) and ethambutol (EMB) for two months (intensive phase), followed by the use of INH and RMP for four months (maintenance phase) (Brazil, 2011). Hepatotoxicity is the most commonly reported adverse event, with an incidence of 2 to 28%. This adverse event may be identified by increased aminotransferase levels by up to threefold if accompanied by symptoms or a more than fivefold increase even in the absence of symptoms (Tostmann *et al*., 2008).

INH is the core drug due to its potent bactericidal activity compared to other drugs. The elimination of INH is mainly by hepatic metabolism generating several

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metabolites, including some potentially hepatotoxic metabolites (Isoniazid, 2008; Tostmann *et al.*, 2008).

Tuberculosis, although severe, has a high chance of cure if the treatment is performed correctly; however, the long treatment, adverse effects, such as hepatotoxicity, and other factors can lead to abandonment of the therapy.

One of the factors strongly associated with treatment withdrawal is alcoholism, whose relationship can be verified from the literature data. The study by Costa *et al.* (2014) showed that among 301 patients, 47.7% used alcoholic beverages. Silva *et al.* (2014) evaluated the factors that led to nonadherence to the complete treatment, and alcoholism was mentioned as one of the causes. Additionally, the study by LoBue and Moser (2003) found poorer treatment success rates when associated with excessive alcohol consumption. The review by Lopes *et al.* (2014) found ethylism to be a factor related to treatment withdrawal in five out of 11 studies, and almost half of the studies reviewed.

The study by Moro *et al.* (2016) evaluated factors associated with noncompletion of latent tuberculosis infection treatment in the USA and Canada. Alcohol-abuse patients showed higher rates of withdrawal, with or without the incidence of adverse events. In the same study, researchers found a 2.2-fold higher chance of treatment withdrawal in alcohol-abuse patients than in nondrinkers.

Ethylism is not only an important factor in patient withdrawal but is also associated with an increased risk of tuberculosis. The review by Lönnroth *et al.* (2008) found that approximately 20% of TB patients abuse alcohol and that there is a threefold greater risk of developing the disease when there is alcohol misuse.

The metabolism of ethanol is mainly hepatic through the alcohol dehydrogenase enzyme; however, 10% of the metabolism occurs through the Microsomal Ethanol Oxidation System (MEOS), where the microsomal CYP450 system oxidizes ethanol, mainly through the CYP2E1 isoform, the most efficient to carry out the oxidation of ethanol to acetaldehyde (Riveros Rosas *et al.*, 1997).

Dattani *et al.* (2004) investigated the influence of alcohol on INH pharmacokinetics. The study was performed in healthy volunteers who received 200 mg of INH for one week, and in one aleatory occasion, they also received alcohol one hour before and every hour for seven hours after INH dose intake. There was no significant difference between the control and ethanol groups, and although alcohol intake lasted for many hours, this could be considered an acute ingestion, perhaps explaining why there were no differences.

Kim *et al.* (2014) studied gamma-glutamyl transferase (GGT) as a sensitive marker for early ethanol-induced liver injury in rats. The animals were divided in exposure for 3 days or 5 weeks, and 22% or 38% ethanol (v/v)/kg was administered via gavage. The GGT levels were time- and dose dependent, and the highest levels were found in the group receiving 22% ethanol for 5 weeks.

Considering that CYP2E1 is induced by its substrates (Cederbaum, 2012), ethanol can be metabolized by it and the metabolism of INH by this enzyme leads to the formation of hepatotoxic species, there is a probable pharmacokinetic interaction when there is concomitant use of alcohol during the treatment of tuberculosis.

The aim of this study was to evaluate the effects of repeated exposure to ethanol on the pharmacokinetic parameters of INH in a group of animals treated with INH + RMP + PYR after a single administration of the combination.

MATERIAL AND METHODS

Chemicals

INH standards were obtained from Sigma–Aldrich (St. Louis, MO, USA), oxazepam was obtained from Farmasa (São Paulo, SP, Brazil), and cinnamaldehyde (CA) was purchased from Merck (Rio de Janeiro, RJ, Brazil). Ethyl acetate was acquired from Macron Chemicals (Mexico City, Mexico). HPLC-grade acetonitrile and methanol were purchased from J.T. Baker (Mexico City, Mexico), ultrapure water was obtained from a Millipore Milli-Q system, and formic acid was acquired from Scharlab S. L® (São Paulo, SP, Brazil), and the ethanol administered to the animals was purchased from Qhemis (Frontignan, France).

Methanol was used to prepare stock solutions of INH (100 µg/mL), oxazepam (1 mg/mL) and cinnamaldehyde (1% v/v).

Animals

Male Wistar rats (weighing 220-270 g) were housed in an environmentally controlled room (23 ±1°C, 55± 5% relative humidity) and light cycle (12/12) with water and food *ad libitum* except before the administration of the drugs, when the animals went through 12 h of fasting. The experiments were performed in the light phase. The preclinical study protocol was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences, UNESP, Araraquara (process 98/2015).

Experimental protocol

The animals in the control group (n=6) received 10 mL/kg 8% glycosidic solution via gavage for three days (Quinteros, 2013). The ethanol group (n=6) received 2 g/kg of 20% glycoalcoholic solution via gavage for three days (Quinteros, 2013). For either group, on the third day, the animals underwent surgery for cannula implantation in the femoral artery, allowing serial blood collection. On the next day, after 12 hours of fasting, oral administration (gavage) was performed at doses of 100 mg/kg RMP, 350 mg/kg PYR and 100 mg/kg INH, with a 15-minute interval between each drug administration (Figure 1). The doses of INH and RMP used were based on the study of Yue (2004), whereas the dose of PYR was based on the study of Baldan (2007). Due to the insolubility of RMP and PYR in water, a suspension of 0.5% carboxymethylcellulose (CMC) was prepared for oral administration. To standardize the absorption of the three drugs, INH was also administered as a suspension.

An aliquot of 250 µL of blood was collected at 0.8, 0.25, 0.5, 1, 1.5, 3, 6, 9 and 12 hours postdose. Plasma was obtained by centrifuging blood samples at 2690 g for 3 minutes and stored at -80°C until analysis.

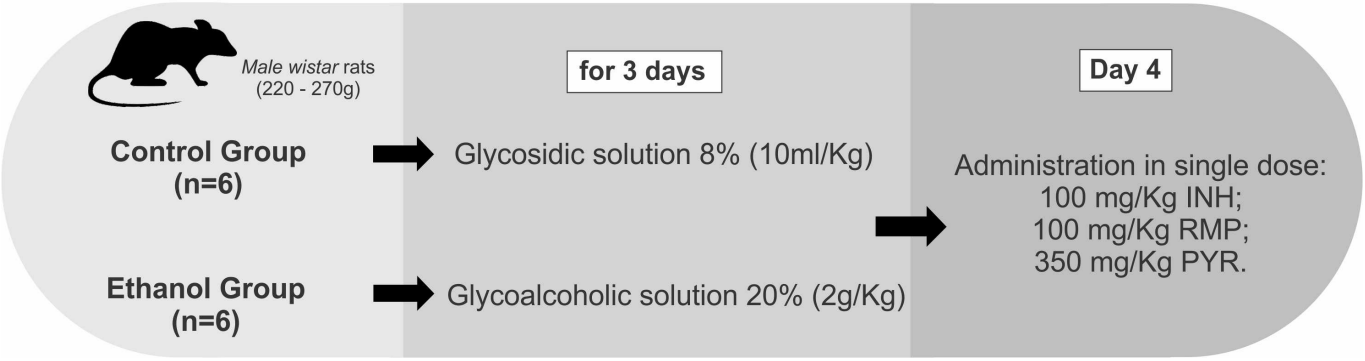

FIGURE 1 - Experimental protocol of administration.

Biochemical parameter assays

The enzymatic activities were assessed pre- and postexposure. Preexposure blood was collected by tail snip, and postexposure samples were collected 12 hours after the administration of the drug combination by the femoral artery implanted cannula.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) kits were purchased from LabTest® Diagnostica SA (Lagoa Santa, Minas Gerais, Brazil).

UHPLC Conditions

The analysis of INH was performed in a chromatographic system consisting of a UPLC Acquity® Waters with a UV–Visible Detector at 340 nm. The separation was carried out in an Acquity® HSS T3 (2.1 ×100 mm; 1.7 µm) column with an HSS VanGuard® (2.1 ×5 mm; 1.7 µm) precolumn. The mobile phase used was methanol, acetonitrile and formic acid 0.2% (05:25:70, v/v/v) in isocratic mode, with a 0.5 mL/min flow rate and run time of 13 minutes. Samples were maintained at

10°C until injection, while the column was maintained at 40°C, and the injection volume was 2 µL.

Sample Preparation

To 100 µL of plasma, 25 µL of IS (oxazepam, 100 ug/mL) was added, followed by 1000 µL of ethyl acetate. The mixture was vortexed and then centrifuged at 7780 g for 10 minutes at 4°C. Then, 900 µL of the supernatant was placed in a new microtube, another 1000 µL of ethyl acetate was added to the mixture, and 900 µL of supernatant was collected after vortex mixing and centrifugation. A total of 1800 µL of the supernatant was evaporated in a vacuum evaporator for 40 minutes at 40°C, and the residue was resuspended in 100 µL of the mobile phase.

Following the method developed by Seifart *et al.* (1995), derivatization of INH with cinnamaldehyde (CA) was used. For that, 10 µL of water and 20 µL of 1% CA were added to the resuspended residue. After homogenization, the sample was kept at room temperature for 10 minutes. Samples were filtered with PTFE syringe filters (0.22 µm) into vials that were placed in the chromatographic system for analysis (Figure 2).

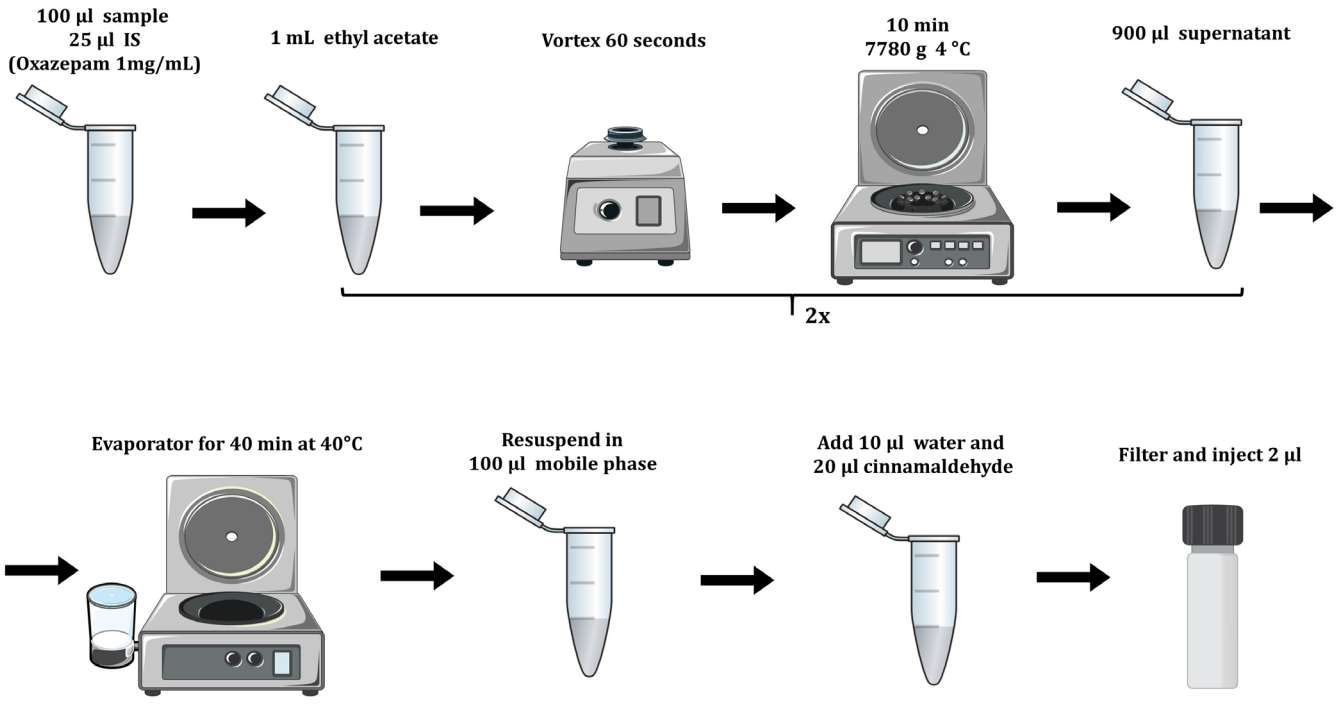

FIGURE 2 - Sample preparation and bioanalysis of INH in plasma.

Bioanalytical method validation

This method was developed and validated according to ANVISA resolution 27/2012 and the Guidance for Industry (2001) US Food and Drug Administration (Brazil, 2012; FDA, 2001). The calibration curve range was from 0,078 to 20 µg/mL, and the correlation coefficient (r) was 0.997, indicating the linearity of the method, since the value of r must be at least 0,98. The precision and accuracy parameters were evaluated for the limit of quantification and quality controls (high, medium, low and diluted) in intra- and interday runs. Precision presented values between 2,84-14,24% for the intraday and 9,62-16,78% for interday. The acceptance criteria

were up to 15% for general controls and up to 20% for the low-quality control, which presented 16% variation. Intraday accuracy showed values between 85,98-114,41% and interday values of 95,51-113,89%, within acceptance criteria, since a variation between 85 and 115% is allowed.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated based on the plasma concentration vs time curves. The elimination half-life ($t_{1/2}$) was determined by the slope of the elimination phase of the semilogarithmic curve. The absorption half-life ($t_{1/2}a$) was determined by the method of residuals. The elimination (Kel) and absorption (Ka) constants were calculated by the formula $0.693/t_{1/2}$ or $t_{1/2}a$. Ka was used to calculate the mean absorption time (MAT) by the formula 1/Ka. The area under the curve from 0 to the last quantifiable concentration (AUC0–t) was calculated by the trapezoidal method, and the area under the curve from 0 to infinity (AUC0-∞) was calculated by the formula AUC0–t + (Cn/Kel), where Cn was the last quantifiable plasma INH concentration. The area under the moment curve (AUMC) was calculated by the statistical moments method and was used to determine the mean transit time (MTT); MTT= AUMC/AUC0–∞. The mean residence time (MRT) was calculated by the equation MRT= MTT – MAT. The clearance (Cl/f) and the distribution volume (Vd_{area}/f) were determined by the equation Cl/f= dose/AUC0–∞ and Vz/f=Cl/f/kel. The maximum drug plasma concentration (Cmax) was obtained directly from the experimental data, as was the time of the occurrence of Cmax (Tmax). The lag time for absorption (t_{lag}) was calculated using the equation t_{lag}=Ln(A/B)/(A-B), where A is the y-axis intercept of the residual line (absorption line) and B is the y-axis intercept of the elimination line. The formulas described were inserted in Excel software for the calculation of pharmacokinetic parameters, and values were confirmed by Phoenix WinNonlin software.

Statistical analysis

Pharmacokinetic parameters are expressed as the mean +/- 95% confidence interval (95% CI). The pharmacokinetic parameters between groups were compared using the nonparametric Mann–Whitney test. Biomarkers were compared using the nonparametric paired Wilcoxon test and nonparametric Mann–Whitney test (GraphPad Instat software, version 3.06).

RESULTS AND DISCUSSION

The pharmacokinetic profiles of isoniazid administered to the control and ethanol groups are shown in Figure 3 and Table I. The pharmacokinetic parameters for both groups are shown in Table II.

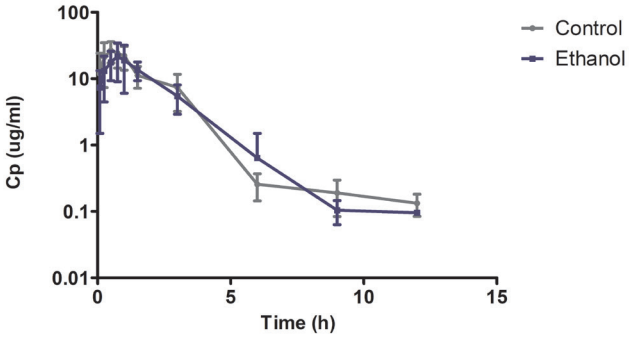

FIGURE 3 - Plasma concentration versus time profile of INH (100 mg/kg) in Wistar rats (male, 250 g) after 3 days administration of (circle) glycosidic solution (n =6) and (squares) glycoalcoholic solution (n = 6).

TABLE I - Plasma concentrations of INH in Wistar rats after administration of 100 mg/Kg INH, 350 mg/Kg PYR and 100 mg/Kg RMP by gavage to the experimental groups: control and ethanol (n=6 in each group). Data are expressed by mean (CI95)

Time (h)	Control Cp (ug/mL) – CI95	Ethanol Cp (ug/mL) – CI95
0.08	12.81 (2.09 – 23.53)	7.36 (1.21 – 13.50)
0.25	21.61 (9.94 – 33.28)	13.15 (4.05 – 22.25)
0.50	25.14 (16.39 – 33.89)	17.64 (8.95 – 26.33)
0.75	22.98 (14.26 – 31.70)	21.77 (8.41 – 35.13)
1.00	21.01 (13.3 – 28.70)	19.04 (5.37 – 32.70)

TABLE I - Plasma concentrations of INH in Wistar rats after administration of 100 mg/Kg INH, 350 mg/Kg PYR and 100 mg/Kg RMP by gavage to the experimental groups: control and ethanol (n=6 in each group). Data are expressed by mean (CI95)

Time (h)	Control Cp (ug/mL) – CI95	Ethanol Cp (ug/mL) – CI95
1.50	11.11 (7.70 – 14.52)	13.63 (9.14 – 18.12)
3.00	6.77 (2.83 – 10.70)	5.48 (2.79 – 8.16)
6.00	0.24 (0.14 – 0.34)	0.64 (-0,72 – 2.00)
9.00	0.18 (0.089 – 0.27)	0.10 (0.0018 – 0.20)
12.00	0.13 (0.091 – 0.17)	0.096 (0.096 – 0.096)

Control group: received an 8% glycosidic solution via gavage (10mL/Kg) for three days. Ethanol group received a 20% glycoalcoholic solution via gavage (2g/Kg) for three days. There was no statistically significant difference between the plasma concentrations of the control and ethanol group for each time (Mann-Whitney test; $p>0.05$).

TABLE II - Pharmacokinetic parameters of INH after oral administration (100mg/kg) in experimental groups: control (n=6) and ethanol (n=6)

Parameters	Control Group	Ethanol Group
	Mean (±CI 95%)	
AUC 0-inf (ug/mL.h)	56.64 (36.50-76.78)	47.44 (35.56-59.33)
Kel (h^{-1})	0.52 (0.45 – 0.59)	0.81* (0.58 – 1.03)
$t_{1/2}$ elimination (h)	1.34 (1.15 – 1.54)	0.91* (0.57 – 1.24)
Cl/F (mL/h/Kg)	1934.90 (1255.20 – 2614.60)	2190.00 (1752.10 – 2627.90)

TABLE II - Pharmacokinetic parameters of INH after oral administration (100mg/kg) in experimental groups: control (n=6) and ethanol (n=6)

Parameters	Control Group	Ethanol Group
	Mean (±CI 95%)	
Vd/F or Vz(mL/Kg)	3883.70 (1908.9-5858.5)	2959.93 (1682.8-4237.1)
Vc/F (mL/kg)	4079.69 (2249.5-5909,9)	2603.62 (932.70-4274.6)
Cmax (ug/mL)	30.19 (22.14 – 38.24)	23.65 (10.86 – 36.45)
Tmax (h)	0.58 (0.31 – 0.85)	0.75 (0.28 – 1.21)
Ka (h^{-1})	9.60 (2.17 – 17.07)	3.65 (0.81 – 6.49)
$t_{1/2}$ a (h)	0.16 (0.034 – 0.29)	0.26 (0.127 – 0.389)
MAT (h)	0.24 (0.048 – 0.46)	0.37 (0.18 – 0.56)
MTT (h)	1.79 (1.49 – 2.10)	1.72 (1.18 – 2.26)
MRT (h)	1.56 (1.32 – 1.79)	1.35 (0.88 – 1.8)
t_{lag} (h)	0.04 (0.02 – 0.05)	0.03 (0.009 – 0.05)

Control group: received an 8% glycosidic solution via gavage (10mL/Kg) for three days. Ethanol group received a 20% glycoalcoholic solution via gavage (2g/Kg) for three days.AUC 0-∞: area under concentration-time curve from 0 to the last quantifiable concentration from 0 extrapolated to infinity; Kel: elimination constant; t1/2: elimination half-life, Cl/F: apparent clearance, Vd/F: apparent volume of distribution, Vc/F: central volume of distribution; Cmax: maximum plasma concentration and Tmax: time of occurrence of Cmax.; Ka: absorption constant; t 1/2 a: absorption half-life; MAT: mean absorption time; MTT: mean transit time; MRT: mean residence time, tlag: lag time for absorption *$p< 0.01$, Mann-Whitney test.

TABLE III - Biochemical parameters in Wistar rats after administration of 100 mg/Kg INH, 350 mg/Kg PYR and 100 mg/Kg RMP by gavage to the experimental groups: control and ethanol (n=6 in each group). Data are expressed by mean

	Control Group		**Ethanol Group**	
	ALT (U/L)	*AST(U/L)*	*ALT (U/L)*	*AST(U/L)*
Pre-exposure	71,94	183,4	49,61	112,7
Post association	81,76	437,1[a]	83,86[a]	316,2[a]
Reference Values	*ALT (U/L)*		*AST (U/L)*	
Branco et al, 2011	62		277	
Giknis and Clifford, 2008	18-45		74-143	
Olfert,Cross & McWilliam, 2017	17-50		39-92	

There are no statically difference between the control and ethanol groups.
a: $p<0,05$ when compared to pre exposure, Wilcoxon matched-pair test.

The INH pharmacokinetics profile was best described by the one-compartment model in both groups. The elimination constant (Kel) and the elimination half-life (elimination $t_{1/2}$) presented a statistically significant difference between the control and ethanol groups. The ethanol group presented a higher Kel (0.81 vs 0.52 h^{-1}) and therefore a lower elimination $t_{1/2}$ (0.91 vs 1.34 h). The Kel reflects the rate at which this process occurs, and it is directly related to the elimination $t_{1/2}$, which, in turn, is a hybrid parameter that is directly proportional to distribution volume (Vd) and inversely proportional to clearance (Cl); that is, a lower Vd or a higher Cl leads to a lower elimination $t_{1/2}$.

At first sight, it may seem that the decrease in the elimination $t_{1/2}$ in the ethanol group may be due to a possible induction of CYP2E1 by ethanol (Riveros Rosas *et al.*, 1997; Cederbaum, 2012), which led to an increase in the metabolism of INH (Tostmann *et al.*, 2008). Another hypothesis to explain the decrease in the elimination $t_{1/2}$ of INH in the ethanol group is that it may have occurred due to increased blood flow and diuresis caused by ethanol (Brunton *et al.*, 2011), resulting in a faster elimination of the drug.

Cl is the pharmacokinetic parameter that describes the ability of the body to remove the drug from systemic circulation, either by metabolism or excretion of the unchanged compound. The Cl values express the volume of biological fluid that is cleared of the drug per time unit. Thus, Cl can be influenced by blood flow, cardiac output and enzyme activity in metabolizing organs. The hypothesis that the decrease in elimination $t_{1/2}$ results from increased metabolism or excretion of INH should be accompanied by increased CI, which was not observed in our study (1934.9 vs 2 190.0 mL/h/kg).

Another relevant aspect to be evaluated relates to the metabolism of INH. The role of CYP2E1 in the metabolism of INH is to catalyze the formation of hepatotoxic species. In the present study, although there was an increase in biomarkers after exposure to the drug combination, no difference between the groups was observed (Table III and Figure 4), and if there was metabolic induction, this induction was not high enough to alter the elimination of INH or to increase the liver biomarkers evaluated. The study of Yue *et al.* (2004) found that after the administration of INH (100 mg/kg; intraperitoneal) for 10 days in Wistar rats, the ALT and AST enzymes were not altered, but there was a nearly fourfold increase in CYP2E1 activity in the same period.

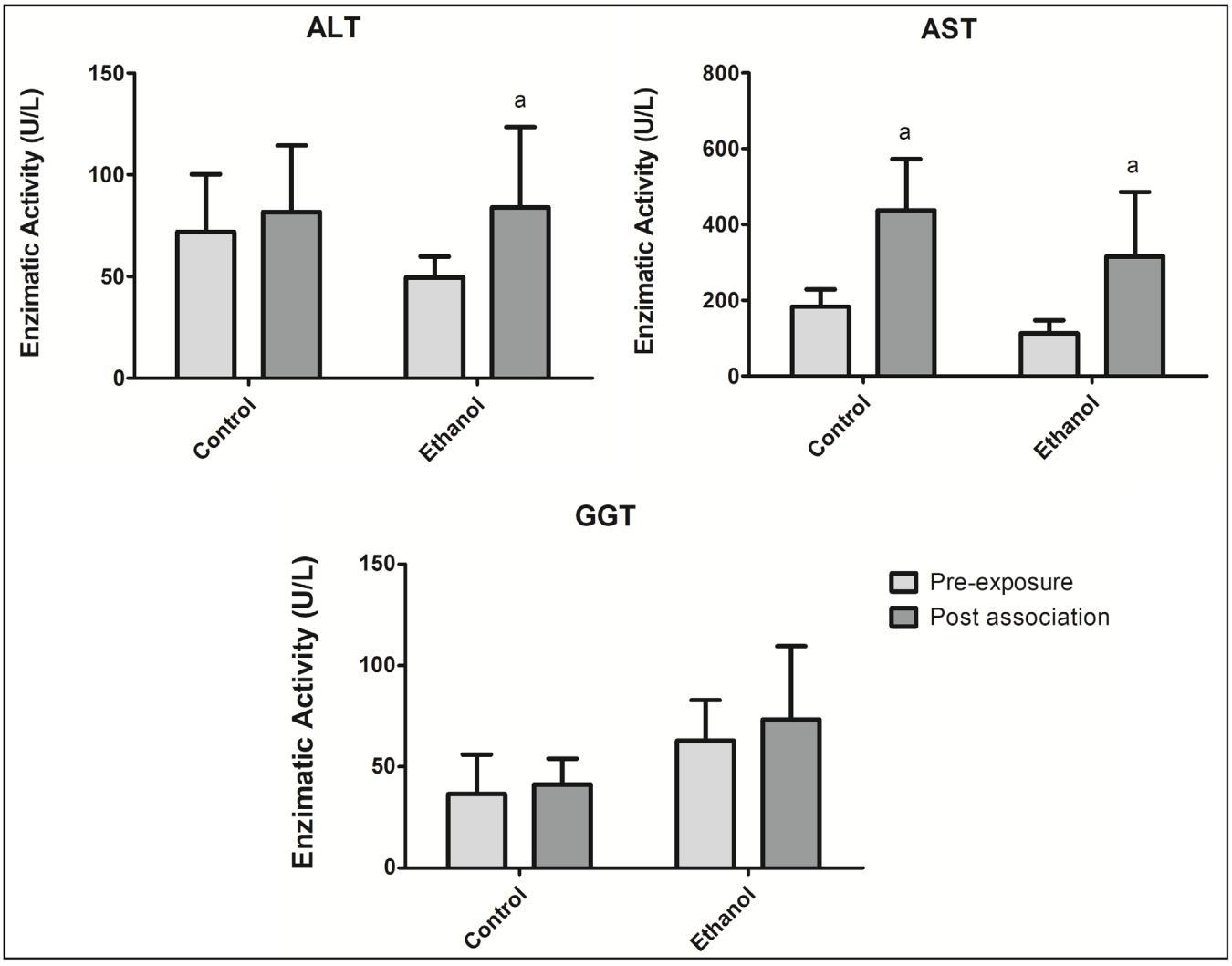

FIGURE 4 - Enzymatic activity of ALT, AST and GGT. Pre exposure indicates the enzymatic activity prior to any exposure; post exposure indicates the enzymatic activity after the administration of glucose or ethanol (control and ethanol group, respectively), and post association indicates the enzymatic activity after the single administration of the drug association. There was no significant difference between the control and ethanol groups. a: $p<0,05$ compared to preexposure, Wilcoxon matched-pair test.

As stated earlier, the elimination $t_{1/2}$ is a pharmacokinetic parameter that relates to Cl and Vd, and if there were no differences in Cl, changes in Vd could be related to the lower elimination $t_{1/2}$ observed in the group of animals that received ethanol.

Vd is the kinetic parameter that can be interpreted in terms of drug distribution and that reflects the capacity of extravascular accumulation of the drug.

By definition, Vd should only be regarded as a proportionality constant between the total amount of drug in the body and plasma concentrations, and as plasma concentrations vary over time, there are several volumes of distribution that can be measured following administration of a drug in an organism. Three commonly calculated Vd are the volume of the central compartment (Vc), the volume of distribution calculated by the area method (V_{area} or Vz) and the steady-state volume of distribution (Vss).

Although the Vd parameter is often used to assess the extent of extravascular accumulation of a drug, the comparison of Vc and Vz, for instance, may assist in the assessment of the rate of distribution, which may be relevant for understanding the time of occurrence of the maximum effect of a drug. Thus, not only the

extent of drug distribution but also the rate at which this distribution occurs are relevant. If the drug is present only in blood, the volume of distribution value will be approximately the total blood volume of the body, and the maximum effect can be observed at the same time as the maximum plasma concentration occurs (Tmax). If the drug reaches extravascular compartments, the rate and magnitude of this spread will determine the intensity and time of occurrence of its maximum effect.

A large Vd results in longer elimination $t_{1/2}$. This means that the plasma levels of the drug are low and the delivery of the drug to the eliminating organs from the bloodstream occurs more slowly. The Vd parameter can be influenced by blood flow, cardiac output, changes in plasma protein binding and body composition.

Thus, the decrease in elimination $t_{1/2}$ in the ethanol group should be accompanied by decreased Vd. Vz/f was calculated, and no significant difference was observed between groups (3883.68 vs 2959.93 mL/kg). The absence of a significant difference between the groups for the Cl and Vd parameters did not show the origin of the lower elimination $t_{1/2}$ observed in the group exposed to ethanol.

It should be noted that the apparent clearance (Cl/F) and the apparent volume of distribution (Vd/F) rather than absolute Cl and Vd have been determined (Toutain, Bousquet-Mélou, 2004). As in the present protocol, only oral administration was performed, it was not possible to determine the bioavailability.

Differences in the extension of absorption may have consequences on Cl and Vd, and although the absolute bioavailability has not been calculated, it is possible to compare the relative bioavailability of INH between the groups through the area under the concentration-time curve (AUC). This mathematical tool, calculated by the integral of the concentrations over time, expresses the extent of exposure of the organism to the drug after administration. This parameter did not present a significant difference between the control and ethanol groups (56.64 vs 47.44 ug/mL.h), indicating that the extent of INH absorption was similar in both groups.

Other absorption parameters related to the absorption rate, either directly (Ka, $t_{1/2}a$, t_{lag}, and MAT) or indirectly (Cmax and Tmax), were evaluated and compared. None of these parameters presented significant differences between the control and ethanol groups, demonstrating that there was no difference in the INH absorption rate.

Mitchison 1998 described mechanisms of resistance to tuberculosis treatment, and among these mechanisms is the occurrence of subinhibitory concentrations during regrowth of the bacilli population. This mechanism is particularly applicable to isoniazid. Therefore, the occurrence of minimal inhibitory concentrations is as important as maintaining these concentrations during the regrowth phase of the bacterial population. Thus, the lower isoniazid elimination half-life may have an impact on the clinical outcome of the treatment of tuberculosis if exposure to ethanol generates differences in its mean time parameters.

The mean time parameters allow the assessment of how long the drug remains in the body unchanged. These parameters (mean transit time - MTT and mean residence time - MRT) express the average period that the drug molecules (unchanged) take to move through the body from the beginning of its introduction, including absorption (MTT), or after the absorption has practically finished (MRT).

MTT and MRT were not significantly different between the groups studied, indicating that the rate of the movement of INH was similar and that plasma levels, after the absorption phase, were maintained similar for a similar period of time.

Thus, it was possible to verify a lower elimination half-life of INH in animals pre-exposed to ethanol, although the origin of this difference was not evidenced.

The elimination half-life is commonly used to select dosing intervals, which determines the plasma concentration oscillation as well as the drug accumulation. This relationship will also determine the minimum plasma concentration of the drug. Therefore, the concern about the possibility of ineffective concentrations of isoniazid when there is a chronic use of ethanol is legitimate.

Although the origin of the lower elimination half-life has not been demonstrated, this result points to the risk of occurrence of lower than expected INH plasma concentrations when there is chronic exposure to ethanol, which can lead to subtherapeutic levels and ineffectiveness of the tuberculosis treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

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