

SCINTIGRAPHY OF THE HEPATOBILIAR SYSTEM IN PATIENTS WITH PULMONARY TUBERCULOSIS

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ABSTRACT: The objective of this paper was to evaluate the hepatobiliary function of patients with pulmonary tuberculosis under triple treatment, using the technetium-99m-DISIDA (^{99m}Tc -DISIDA) hepatobiliary scintigraphy. Ten men and three women with pulmonary tuberculosis were subjected to hepatobiliary scintigraphy at the beginning of triple treatment (M1) and two months after it (M2). Patients were from the urban area, of low socioeconomic level, malnourished, and chronic alcohol and/or tobacco users. Ten normal individuals were evaluated as controls. Radiotracer images were acquired on a computerized gamma camera (Orbiter-Siemens) and $T_{1/2}$ uptake and excretion values were calculated. Nutritional status and serum hepatic enzyme levels for each patient were evaluated at M1 and M2. None presented clinical or laboratory antecedent of hepatobiliary disease. At M1, there were no hepatic serum or kinetic alterations of the ^{99m}Tc -DISIDA. At M2, patients presented better nutritional conditions than at M1; there was increased serum aspartate aminotransferase (AST) and reduced excretion time for ^{99m}Tc -DISIDA, which was interpreted as a more adaptive than toxic phenomenon, yet not all alterations were significant and none manifested clinically. Apparently, triple treatment acted on the liver inducing the P450 cytochrome enzymatic system, accelerating radiotracer excretion, which follows the same path as the bilirubins.

KEY WORDS: pulmonar tuberculosis, scintigraphy, hepatobiliar system, antituberculosis drugs.

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INTRODUCTION

Tuberculosis is still a serious public health problem in Brazil. Since the 1970s, the short term triple scheme (six months) with isoniazid (INH), rifampicin (RFP) and pyrazinamide (PRZ) has had considerable success. However, these antituberculosis drugs are potentially hepatotoxic, particularly when associated (1).

Biotransformation of INH mainly occurs in the liver by acetylation by N-acetyltransferase, producing toxic metabolites such as monoacetylhydrazine (20, 21, 22). N-acetyltransferase presents genetic polymorphism with bimodal distribution in the population, documented by the presence of both rapid and slow acetylator individuals (18). The relative frequencies of these phenotypes differ between geographic areas and ethnic groups. To prevent potential adverse manifestations, exposure to antituberculosis drugs should be preceded by determining INH acetylator phenotype. There are indications that rapid acetylators are more susceptible to hepatotoxicity by INH, since they present a greater quantity of N-acetyltransferase and therefore acetylate INH in monoacetylhydrazine more rapidly (18). However, this hypothesis still remains unclarified (12).

Besides genetically determined enzymatic polymorphism, biotransformation of drugs by the liver is influenced by age, alcoholism, tobacco use, nutrition, and advanced-stage tubercular and hepatic diseases; these are considered risk factors that create predisposition to hepatotoxicity from antituberculosis drugs (9, 12, 15, 19, 27).

Iminoacetic acid (IDA) and its analogs labeled with ^{99m}Tc are important radiotracers for exploring hepatocyte function and the perviousness of the biliary tree due to their high hepatic clearance and low dosimetry (14). Although scintigraphy is an important method for functional and structural examination of the liver (23), permitting evaluation of hepatic diseases, no studies exist in literature using scintigraphy to evaluate hepatocellular function in patients subjected to triple antituberculosis therapy. This work aims to evaluate hepatic alterations, which can occur during a treatment that has potentially toxic action on the liver, using the hepatobiliary scintigraphy.

PATIENTS AND METHODS

This study was approved by the Research Ethics Committee of the Botucatu Medical School, São Paulo State University. Thirteen pulmonary tuberculosis patients were studied. They were hospitalized at the University Hospital between July 2001 and

September 2002. There were 10 males (77%) and 3 females (23%) with mean age of 43 ± 12 years, ranging from 21 to 59 years, of low socioeconomic level (77%), alcohol drinkers (61%) and/or tobacco users (92%), and rapid (45%) and slow acetylators (55%). Ethnicity was: nine whites (69%), two mixed-race (15%), and two blacks (15%). Each patient answered a specific questionnaire on clinical history and socioeconomic profile.

Exclusion criteria were: those who did not sign the informed consent form; those who were pregnant; minors under eighteen years old; those in treatment or with a previous history of treatment for tuberculosis; and those with serological reaction to human immunodeficiency virus (HIV), to surface antigen of B virus (HBsAg), or to the hepatitis C virus (HCV).

Immediately after diagnostic confirmation of tuberculosis, and before initiation of triple treatment – considered the start of the study (M1), all patients were subjected to clinical-nutritional evaluation, examinations of urine, serum and hematological biochemistry, and hepatobiliary scintigraphy. These exams were repeated two months after the start of triple treatment, when PRZ was withdrawn – considered the second moment of the study (M2). HIV, HCV, HbsAg serologies and acetylator phenotype determinations – colorimetric test *in vitro* (10, 13) – were only made at M1.

Scintigraphic evaluation of the hepatobiliary system was preceded by a 2-8h fast. The standard dose of the radiopharmaceutical ^{99m}Tc -DISIDA (IPEN, USP, São Paulo) was 180MBq (5mCi) [for adults of approximately 70kg]. Images of the anterior abdomen were taken immediately after intravenous administration of the radiotracer, with patients on a supine position. A low-energy and general-purpose collimator coupled to a computerized gamma camera (Orbiter, Siemens, Germany) was used. The dynamic study was recorded on a 64X64 matrix, with 60 images every 60 seconds, performed during 60 minutes on each patient. Images were processed on an ICON computer, software version 7.5 (Siemens - Germany). Regions of interest (ROIs) were drawn over the right superior projection of the liver and on the heart and time-activities curves were generated. $T_{1/2}$ uptake ($T_{1/2\text{Upt}}$) and $T_{1/2}$ excretion ($T_{1/2\text{exc}}$) values for ^{99m}Tc -DISIDA were calculated using an exponential adjustment of the curves by the least squares method (3). Ten healthy volunteers (controls) underwent scintigraphic evaluation of the liver and biliary tree at one moment (Table 1).

Mean (X), standard deviation (SD), and coefficient of variation (CV) were calculated for each variable at M1 and M2. Comparison between moments M1 and M2 was performed by the *t* test for two paired samples (dependent) with calculations for *t* and *p* (8). The relationship between variables at each time was estimated by the Pearson correlation coefficient between pairs of variables. Results were considered significant when $p < 0.05$; cases in which $0.05 < p < 0.10$ were assumed to have a tendency toward significance (8).

RESULTS

Patients showed significant improvement between the two studied times; mean M2 values for body mass index (BMI), and red corpuscle, hemoglobin and hematocrit levels were higher than at M1 (Table 1). From mean hepatic serum enzyme values, only AST was significantly higher at M2 than at M1; the other hepatic enzymes were higher at M2, but not significantly (Table 2).

The mean $T_{1/2}$ uptake of ^{99m}Tc -DISIDA in the control group (CG) was significantly higher than in pulmonary tuberculosis patients at M2 (Table 3). In pulmonary tuberculosis patients, there were no significant differences of mean $T_{1/2}$ uptake value between M1 and M2 ($M1=M2$). For $T_{1/2}$ excretion, there was no significant difference between CG and patients, but at M2 the patients had a lower mean $T_{1/2}$ excretion value than at M1.

Table 1: Nutritional status and hematometric values ($X \pm SD$) of patients with pulmonary tuberculosis at both study moments (M1, before triple treatment; M2, two months after treatment initiation).

	Weight (Kg)		BMI (kg/m ²)		WBC (10 ³ /mm ³)		RBC (10 ⁶ /mm ³)		Ht (%)		Hb (g%)	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
X	55.3	61.9	20.49	23.08	9.08	7.67	4.53	5.06	35.92	42.0	11.50	13.74
SD	10.2	12.9	3.6	4.54	2.95	2.89	0.48	0.74	4.25	4.57	1.67	1.54
Statistics	t=3.65 p<0.001		t=4.29 p<0.001		t=1.783 p>0.10		t=2.906 p<0.02		t=5.638 p<0.001		t=5.332 p<0.001	
Comments	M1<M2		M1< M2		M1=M2		M1<M2		M1<M2		M1<M2	

BMI : Body mass index; WBC: White blood cell; RBC: Red blood cell; Ht: Hematocrit; HB: Hemoglobin.

Table 2: Serum biochemical parameters (X±SD) of patients with pulmonary tuberculosis at both study moments (M1, before triple treatment; M2, two months after treatment initiation).

	AST (4-20mUi/ml)*		ALT (2-18mUi/ml)		ALP (3-126 mUi/ml)		Gamma-GT (15-73 mUi/ml)	
	M1	M2	M1	M2	M1	M2	M1	M2
	X	13.00	23.55	14.15	20.77	110.92	133.31	66.0
SD	7.85	18.11	14.32	26.48	46.23	75.74	45.67	102.47
Statistics	t=2.659		t=0.934		t=1.020		t=1.296	
	p<0.05		p>0.30		p>0.30		p>0.20	
Comments	M1<M2		M1= M2		M1=M2		M1=M2	

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; Gamma-GT: Gamma glutamyl transpeptidase; *: Reference values from local laboratory.

Table 3: T_{1/2} uptake and T_{1/2} excretion (X±SD) of DISIDA [^{99m}Tc] of controls (CG) and patients with pulmonary tuberculosis at both study moments (M1, before triple treatment; M2, two months after treatment initiation).

Groups and moments	T _{1/2} Uptake (minutes)	T _{1/2} Excretion (minutes)
CG (n=10)	2.43±0.87	24.61±6.93
M1 (n=13)	1.88±0.75	27.66±7.64
M2 (n=13)	1.46±0.62	22.08±4.09
Statistics	CG=M1: p>0.05	CG=M1: p>0.05
	CG>M2: p<0.05	CG=M2: p>0.05
Comments	M1=M2: p>0.10	M1>M2: p<0.05

CG: Control group, normal individuals; Student's *t* test.

DISCUSSION

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (γ -GT) levels, bilirubins, and hepatobiliary scintigraphy were normal at M1. There were significant changes in laboratory-clinical patient profile at M2, indicating improved general state. From the perspective of hepatic integrity and hepatocytic function indicators, there was a significant increase in AST serum activity and a significant decrease in mean $T_{1/2}$ value of ^{99m}Tc -DISIDA excretion at M2; in other words, radiotracer excretion was faster.

Although not specific, AST serum levels are also used as indicators of hepatic disease activity. In light lesions, AST serum elevation is due to the cytoplasmic component, and in grave mitochondrial lesions AST also participates; for this reason, high AST levels indicate complete alteration of hepatocytes (24). Thus significantly augmented AST levels at M2 can indicate hepatocyte alterations, which did not exist at M1, possibly associated with cellular injury and probably dependent on triple treatment. It must be remembered that most of the patients had antecedents of alcoholism, this can enable hepatocyte alterations caused by anti-tuberculosis drugs. This is reinforced by increases (not significant) in other enzymes values that reflect normal hepatocyte, such as ALT and γ -GT, when they are compared to M1 values and reference norms. In summary, analysis of serum enzymatic levels suggests that triple treatment had some effect on the liver, which was apparently more adaptive than toxic as not all alterations occurred in a significant manner and none manifested clinically.

The enzymatic system associated with P450 cytochrome is the main responsible one for drug biotransformation in the human organism, and is highly concentrated in the smooth endoplasmic reticulum, microsomal hepatocyte fraction. Lewis *et al.* (16) suggested that enzymatic alterations occurring during treatment with drugs that induce microsomal enzymes or act on bilirubin metabolism probably translate to an intermediate stage between adaptation and hepatic toxicity. A very potent inducer for this system is phenobarbital (11); rifampicin and isoniazid are also recognized inductors (5). Apparently, rifampicin acts at various levels of organic anion hepatic transport and, in an analogous manner, radiotracers can compete with excretion of bilirubins (6). Even with prolonged treatment, rifampicin is associated with asymptomatic enzyme increase, which can be ignored unless the patient presents

some clinical manifestation indicative of hepatic lesion (6). Therefore patients from this study could have presented a stimulated hepatic enzymatic system at M2.

The question of whether the acetylator phenotype creates a predisposition for hepatitis development due to medicaments is controversial. In the Brazilian population, the distribution of hydrazide acetylation phenotypes occurs in equal parts, with about 50% rapid and 50% slow acetylators (2), which agrees with patients in this work, in which 45% were rapid acetylators and 55% slow. In this study, of the six slow-acetylator patients, two presented increases in almost all hepatic enzymes at M2, while from the five rapid-acetylator patients, only one had increases in all hepatic enzymes at M2. Although these results tend to agree with those of Gronhagem-Riska *et al.* (12) and Martinez-Roig *et al.* (18), in which individuals with slow acetylator phenotype are more predisposed to develop hepatotoxicity, the small sample size does not permit a conclusion on the relation between acetylator phenotype and hepatotoxicity dependent on triple treatment.

The iminodiacetic acid derivatives, e.g., DISIDA, used on hepatobiliary scintigraphy are, like bilirubins, organic anions with transit similar to those through the liver and biliary pathway (17, 26). Thus the transport mechanism of bilirubin and the transit of bile, composed of water, inorganic electrolytes, organic anions, biliary acids, lipids, and proteins through the biliary system, are important models for understanding findings from hepatobiliary scintigraphy. At M2, mean $T_{1/2}$ uptake for hepatocytes was not significantly different from M1. Considering the results for hepatic enzymes already discussed and knowing that eventual alterations in $T_{1/2}$ uptake not observed in this study indicated hepatocyte lesions (4), we can reiterate that possibly the alterations found at M2 depended more on a hepatic adaptive process for drugs that stimulated and were metabolized in the liver than on the hepatocyte lesion they induced. The significantly smaller $T_{1/2}$ uptake value of patients with tuberculosis at M2, compared with that of the control group, reinforces the hypothesis that livers of treated individuals were stimulated by triple treatment.

With regard to radiotracer excretion at M2, it is important to emphasize that the average duration of this process remained within normal standards described in literature (26) and did not differ from the mean value in the control group. However, it was significantly smaller than the mean value at M1, indicating an increased excretion velocity at M2 in these patients. When scintigraphic evaluations of the liver and biliary tree with ^{99m}Tc -DISIDA are made after exposure to phenobarbital,

alterations occur in radiotracer distribution and excretion (25). Although no information exists about the effects of triple treatment on bile flow, it cannot be ignored that this effect exists and influences the transit of isotopically labeled organic anions like ^{99m}Tc -DISIDA, since the drugs used in that treatment stimulate the liver similarly to phenobarbital (5, 11). So it is possible that active metabolites formed from biotransformation of these drugs may have stimulated hepatocyte function and, consequently, accelerate radiotracer excretion.

Furthermore, it is interesting that average ^{99m}Tc -DISIDA uptake time at M1 would have positive correlation with bilirubin levels at M1. This is understandable since their transit routes are the same, and there is competition between them for these routes (7). At M1, there was no significant correlation between $T_{1/2}$ uptake and $T_{1/2}$ excretion of ^{99m}Tc -DISIDA. At M2, however, a significant direct correlation was seen between uptake velocities and excretion, i.e., increased excretion velocity appears to have been accompanied by greater radiotracer uptake. As a whole, this positive correlation between uptake velocities and excretion also reinforces that at M2 the liver was stimulated by triple treatment drugs. Our results suggest that scintigraphic evaluation shows functional hepatic alterations after triple treatment for pulmonary tuberculosis.

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