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Full Length Research Paper

Pharmacokinetics of isoniazid in 30 tuberculosis patients in Abidjan, Côte d'Ivoire

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Isoniazid is a major component in tuberculosis (TB) treatment, which is often used in combination or alone as prophylaxis for its high efficiency on bacilli *of Mycobacterium tuberculosis*. It is responsible for an increased risk of developing serious liver side effects involving the prognosis of the patient. Its coinfection with HIV is currently a real public health problem. The purpose of this study was to establish the pharmacokinetic profile of isoniazid in Ivorian patients infected with TB taking a combination containing isoniazid at Abidjan. A cross-sectional analytical and descriptive study on the pharmacokinetics of isoniazid in 30 adults Ivorian patients on TB treatment was conducted. Blood samples at intervals of time were performed by high performance liquid chromatography-ultra violet (HPLC-UV). 30 adult patients were enrolled, with sex ratio M/F = 2. Mean age was 38.67 (18 to 67 years). 56 (7%) were slow acetylators. 14 (46.7%) were treated for pulmonary TB smear negative and 36.67% had a co-infection TB/HIV. A positive correlation was also observed between body mass index (BMI) and Vd (0.444 and p = 0.14) and a negative correlation between BMI and Tmax (-0.399, P = 0.29). The main biological variable influencing pharmacokinetic parameters according to the analysis is the acetylation profile of the patient.

Key words: Pharmacokinetics, Isoniazid (INH), tuberculosis, Abidjan.

INTRODUCTION

Isoniazid (INH) is the most bactericidal molecule active ingredients commonly used against tuberculosis (TB) (World Health Organization (WHO), 2010). Major TB drugs, INH, is often used in combination with other molecules or alone in prophylaxis for its high efficiency bacilli *Mycobacterium tuberculosis* (WHO, 2010). However, if the effectiveness of this molecule is undeniable, its toxicity is often a ransom for its therapeutic success. Indeed, INH is responsible for the onset of serious liver side effects life-threatening to the patient, which is based on its metabolism and bioactivation made by NAT2 (N- Acetyltransferase 2) and CYP 2E1 (Chamorro et al., 2013).

Dosing of isoniazid is traditionally performed by body

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weight to approximate the WHO recommended dose of 5 mg kg-[1] (WHO, 2009). However, in fixed combinations of antiTB including INH, the doses are not exactly in the normal range and can therefore rise plasma levels concentrations above or below therapeutic. The increase or decrease in plasma concentration cause sides effects or harmful bacterial resistance. Finally, isoniazid is associated with hepatotoxicity and peripheral neuropathy, and slow acetylators may be as a result of increased risk of toxicity (Cho et al., 2007). Also, treatment with isoniazid is further complicated by the expression of the polymorphism in the enzyme system involved in the metabolism, including genetic defect on chromosome involved in the metabolism of INH (acetylators slow and rapid acetylators) (Parkins, 1997).

It is therefore important to ensure an exposure of anti-infective agents or appropriate TB patients in a clinical setting. In addition, HIV and tuberculosis are a lethal combination. Tuberculosis is a major cause of death among HIV-positive. It is responsible for approximately 13% of acquired immuned deficiency syndrome (AIDS) deaths in the world (WHO, 2010). In Africa, HIV is the main determinant of the increase in the incidence of TB in the past decade (WHO, 2010). Co -infection HIV whose estimated prevalence by WHO was 38% in 2009 (WHO, 2009), is reported by the authors as influencing parameters pharmacokinetics of isoniazid and is suspected in the treatment failures and re-emerging disease. TB is also the first opportunistic infection in HIV.

The pharmacokinetics of isoniazid in the Ivorian adult patient has never been done in our context because of the difficulty of implementing a simple and inexpensive analytical method but also because of a limited technical platform. The objective of this study is to perform the pharmacokinetic of isoniazid in the practices of a poor country conditions and provide as an appropriate response to therapeutic optimization, but also to assess the acetylation profile of patients in order to intensify the pharmacological basis and optimize the therapeutic monitoring of patients pharmacology.

MATERIALS AND METHODS

Patients

This was a descriptive and analytical prospective study with reference from June, 2012 to April, 2013. People enrolled were adult patients hospitalized in the Department of Pneumology of Cocody Hospital in Abidjan, on TB treatment including INH single dose, taking one tablet in its fixed combination, and having received information and after written informed consent.

Chemicals and reagents

Isoniazid reference substance (European pharmacopoeia ICRS0331) purity 99.8%, Methanol HPLC grade (Chromasolv®), orthophosphoric acid H_3PO_4 (Fluka®), KOH (Sigma Aldrich®), ultra water pure HPLC were kindly provided by the National Laboratory of Public Health (Côte d' Ivoire). White plasma was offered by the National Centre for Blood Transfusion Abidjan.

Sampling

Blood samples were collected on heparin tube after administration of conventional TB treatment according to the weight (4 t 5.45 mg/kg) to fasting. Blood samples were collected, respectively for 30 minutes, 1h, 1h30, 2h, 3h, 4h, 8h and 12 hours after the first oral dosing. The collected blood specimens were centrifuged and plasma was transferred under refrigerated atmosphere at 20°C immediately at the Pasteur Institute in Abidjan Cocody and stored at -80°C for subsequent assay of isoniazid.

Analytical methods

The plasma concentrations of INH were analyzed at the Laboratory of Biochemistry fundamental Pasteur Institute of Côte d' Ivoire by high performance liquid chromatography HPLC–UV (C.E.A.E. Québec, 2009). A technical simple extraction was performed by protein precipitation with 300 µl of methanol in 150 µl plasma (Roht, 2012). The mixture is then homogenized by a vortex and centrifuged at 15,000 rpm/min for 10 min at 4°C. The methanolic supernatant was collected in a test tube with a volume equal to adjust the pH to 5.5 (400 µl), 20 µl was then injected for analysis. HPLC system (Waters®) with detector UV was added to a column infusion (Symmetry®) C18: 3.5 microns, 4.6 × 75 mm. The mobile phase consisting of 1 M phosphate buffer and methanol is diffused by concentration gradient (50/50 v/v for the first minute and 90/10). The phosphate buffer was obtained by dissolving 3.8 g of phosphoric acid in ultra pure water in sufficient quantity. A flow rate of 0.9 ml/min at 254 nm and a retention time of 2 min was applied. The limits of detection (LOD) and quantification (LOQ) were 0.1 and 15 mg/L. The linear correlation coefficient was 0.995.

Pharmacokinetic calculations and statistical analyses

Linear order 1 noncompartmental model based on a mathematical application graphic was used to determine the pharmacokinetic parameters of isoniazid [extraction coefficient (Ke), half - life ($T_{1/2}$), maximum concentration (Cmax) maximal time (Tmax), children on the curve to 12 h and at infinity (AUC_{0-12}), (AUC_{12-00}), clearance (CL), volume of distribution (Vd)]. The determination of the area in the curve (AUC) by the trapezoidal method (Houin, 1990). The course of the semi -log time curve functions plasma concentrations were made with Microsoft Excel 2007 software and allowed to deduce other parameters. Determining the acetylation profile of the patient was made by the Vivien method and Coll inactivation index I_3 according to the formula:

$$I_3 = \frac{(C_3 + 0{,}6)}{D\left(\frac{mg}{kg}\right)}$$

This was after measurement of INH concentration at 3 h (C3 mg/L) after administration of a dose D of medicament. Rapid acetylators are defined as having an index $I_3 < 0.65$. The curve of plasma concentration versus time and statistical package for social sciences (SPSS) version 20 was used for the statistical analysis of our data. The student t-test and Fischer Snedecor F for comparison of means and correlation test Tau B Kendall and Spearman, significance was set at $p < 0.05$. The statistical analyses were performed with statistical package for social sciences (SPSS), version 15.

Ethical considerations

The study protocol was reviewed and approved by and National

Table 1. Clinical and demographic features of patients with tuberculosis.

Parameter	N=30 (%)
Sex	
Female	10 (33.3)
Male	20 (66.7)
Age (years)	
-40	18 (60.0)
+40	12 (40.0)
Income	
with IGA*	18 (60.0)
Without IGA	12 (40.0)
Weight (Kg)	
+60	16 (53.33)
-60	14 (46.66)
Body mass index (BMI)	19.39 (12.48-24.80)
Dose/kg	4.75 (4.05-5.45)
HIV	
Negative	19 (63.3)
Positive	11 (36.7)
Acetylators	
Slow	17 (56.7)
Rapid	13 (43.3)
Type of TB	
Extra pulmonary	6 (20.0)
Disseminated	14 (46.7)
Pumlonary	10 (33.3)

IGA * income generating activity

Research Ethics Review Committees. All the patients have given their consent.

RESULTS

Patient characteristics

A total of 30 newly diagnosed inpatient adults TB patients with a mean age of 38.67 years (18 to 67 years) were enrolled in the study. Table 1 summarizes details of demographic and clinical features of the participants.

Pharmacokinetics

Well resolved peak of HPLC chromatograms were obtained for all samples. INH concentration after dosing, which is frequently quoted in the literature as a convenient reference point, varied from as low as 1.76 µg/ml to as high as 7.67 µg/ml. At 2 and 3 h post-dose INH plasma concentration of 3 to 5 µg/ml (Peloquin et al., 1996) and 1.5 µg/ml (Schaaf et al., 2005), respectively have been suggested as a required range for optimal bactericidal effects. Univariate regression analyses showed that there was no association ($P > 0.2$) between INH pharmacokinetic parameters and the covariates: weight, sex, type of TB and concomitant drug use.

There was no significant linear correlation between the biological parameters and pharmacokinetic variables with the exception of blood glucose and the Tmax. In effect, the linear correlation coefficient is positive ($p = 0.377$; 0.044) between blood glucose and Tmax. However on the other hand there is also a positive correlation between body mass index (BMI) and Vd (0.444 with $p = 0.14$) and a negative correlation between BMI and Tmax (-0.399 with $P = 0$ is observed, 29). Patients with a high BMI have a high Vd and relatively low Tmax. The main biological variable influencing pharmacokinetic parameters according to the analysis is the acetylation profile of the patient. In fact, this variable is significantly correlated to all pharmacokinetic parameters (except Vd), acetylation is negatively correlated with Tmax - Cmax - $T_{1/2}$ and AUC_{00}. The slow acetylators have Tmax, Cmax, $T_{1/2}$, and AUC_{00} higher than the rapid acetylators patients, while slow acetylators have Ke lower clearance than rapid acetylators. Finally, HIV status did not influence the pharmacokinetic parameters.

DISCUSION

The study on the pharmacokinetics of isoniazid is the first of its kind in Cote d'Ivoire. The main aim of this work was to assess pharmacokinetic data of Ivorian patients with TB. Our work has not used pharmacokinetics software for the analysis and interpretation of biological information on the future of INH in the blood but rather it has used graphic mathematical method to determine the graphics settings pharmacokinetic (Hoin, 1999). Many inherent difficulties were encountered. Apart from the weakness of our sample, we had to cope with the prohibitive cost of reagents and inputs but also to our lack of experience in plasma assay for pharmacokinetics because Côte d'Ivoire is still in its infancy. However, this study has the advantage to show the pharmacokinetic profile of isoniazid for the analytical and methodological feasibility of clinical pharmacokinetic data for patients in an African context to optimize the care of patients for therapeutic monitoring pharmacologique (Peloquin et al., 2002).

The study involved 30 patients, including 20 men (66.66%) and 10 women (33.33%), with a sex ratio = 2. Some authors have reported the same results in male-dominated work in Africa. This predominance of male

subjects could be explained by the fact that TB primarily affects males in accordance with national and international trends (WHO, 2012). The average age of patients was 38 years, with a range of 18 to 67 years. Young adults (18 to 40 years) were the most represented with 73.33%. TB affects young patients still in have a lower average weight (Table 1).

Most clinical and biological data are within normal values (Table 2). However, 56.7% of subjects were slow acetylators, the predominance of slow acetylators in our study is slightly inconsistent with the work of Marquet and others (Marquet, 2004) with estimated 40% proportion of slow acetylators in the black population against 60% of rapid acetylators. The patients studied were mostly treated for pulmonary TB smears as negative 14 (46.67%) (Table 2). The anti-Tb treatment was therefore administered as presumptive without the presence of diagnostic elements because of diagnostic difficulties related to the lack of resources for patients and the equipment facilities in public health system in Côte d'Ivoire. Although some authors required the demonstration of tubercle bacilli in sputum microscopy by culture or histological examination in TB patients before treatment, medical decision becomes complex in this context. 36.67% had a co-infection with TB-HIV. Some authors have reported different proportions in a similar work. Indeed, Wilkins et al. (2011) reported in South Africa a proportion of 15.2% of HIV positive subjects detected in a study of the pharmacokinetic variability of Isoniazid. The proportion of co-infection HIV reported in our study is close to the estimated prevalence by WHO (38%) (WHO, 2004).

The pharmacokinetic parameters (Table 2) are similar to those in the literature. Authors have reported similar results in work targeting the pharmacokinetics of isoniazid. Indeed, Mclleron et al. (2006) reported the following medium: maximal concentration Cmax 3.07 mg/L vs 3.33 mg/L, with a range of 1.82 and 5.66 mg/L; $T_{1/2}$ 1.59 h against 2.39 h, with a range of 1.21 and 2.17 h. Peloquin et al. (2002) obtained the following average, for a population of 24 subjects: 3.14 ± 0.92 C_{max} mg/L, 1.06 ± 0.58 Tmax h and 13.82 ± 6.87 AUC mgl/h for patients receiving 250 mg Isoniazid and Cmax of 3.77 ± 1.11 mg /L, 1.06 ± 0.58 Tmax h and 16.59 ± 8.24 AUC mg.l/h. The volume of distribution (Vd) 0.9 ± 0.27 showed that INH distribution in plasma was correlated with the data found in most publications (Elmendorf et al., 1952). This study has showed that despite the high interindividual variability and intra-individual pharmacokinetic data, plasma concentrations of Ivorian adults subjects are superimposed values of plasma concentrations of other subjects.

Most of our patients have a relatively rapid absorption of isoniazid (0 to 1 h), according to some published earlier in which isoniazid was shown to be rapidly and completely absorbed (Elmendorf, 1952; Des Prez, 1961). Multivariate analysis of pharmacokinetic parameters and linear correlation (Kendall tau b and Spearman's rho) (Table 3) showed no significant differences between age and $T_{1/2}$, AUC and Cmax (P, respectively 0.27, 0.74 and 0.81). The difference between age is significant for Tmax (P = 0.03). In our study, the $T_{1/2}$, AUC and Cmax did not affect age. The Tmax ranged against one age to another. These results suggest that only the time to reach maximum concentration Isoniazid is influenced by age, and is particularly high in the extreme age brackets. Authors have obtained different results by exploring the influence of age on the pharmacokinetics of isoniazid. Indeed, Kergueris (1986) rather highlighted the influence of age on the half-life of elimination. Rey et al. (1998, 2001) has observed the decrease in the half-life when age increased. McIlleron (2006) observed in their work a growth of 6% of the maximum concentration for each additional year. Age can be considered as a prime factor variation in pharmacokinetic parameters of isoniazid.

Our work shows no significant difference between sex and pharmacokinetic factors (Table 3). Indeed, some authors have noted this lack of influence of gender on the pharmacokinetics. McIlleron (2006) reported to this effect no significant difference between the maximum concentrations observed in both sexes (P = 0.104). Thee (2011) had found no significant difference between the famous genres for the following parameters: Cmax, Tmax, AUC (P = 0.742, respectively, 0.083 and 0.476). However, McIlleron et al. (2006) led to the conclusion of its work on the pharmacokinetics of Isoniazid young TB that gender was a factor in convincing risk of failure of TB treatment by interference on Cmax. Substantial variability in absorption kinetics means that the use of a single consistent time point for Therapeutic Drugs Monitoring (TDM) is unlikely to provide a reliable estimate of true isoniazid exposure. In any event, TDM is of limited practical use in resource-poor high-burden countries, where it is currently unavailable and unlikely to become available in the foreseeable future.

Our results also show no significant difference between the patients and biological parameters of the pharmacokinetic variables with the exception of blood glucose and the Tmax (Table 4). In effect, the linear correlation coefficient is positive (p = 0.377 and 0.044). Thus, the plasma concentrations of INH are higher in patients with high blood glucose levels. High blood sugar increases the risk of exposure to INH and increase the frequency of occurrence of side effects. However on the other hand there is also a positive correlation between BMI and Vd (0.444 with p = 0.14) and a negative correlation between BMI and Tmax (-0.399 with P = 0.29). Patients with a high BMI have a significant Vd and relatively low Tmax. The main biological variable that influences the pharmacokinetic parameters analysis by acetylation is the profile of the patient (Table 4). In fact, this variable is significantly correlated to all pharmacokinetic parameters except Vd. The acetylation is negatively correlated with Tmax - Cmax - $T_{1/2}$ and

Table 2. Pharamcokinetics characteristic (Kendall and spermann tests).

Parameter	Tmax (h)	Cmax (µg/ml)	$T_{1/2}$ (h)	Ke (h-1)	AUC_{00}	Clearance ($l.h^{-1}.kg^{-1}$)	Vd (l/kg)
Sex							
----	-0.044	-0.15	0.093	-0.093	-0.048	0.067	0.233
P-value	0.796	0.33	0.548	0.548	0.756	0.663	0.13
Age (Years)							
---	0.072	0.044	-0.109	0.109	-0.095	0.146	-0.026
P-value	0.615	0.734	0.401	0.401	0.464	026	0.844
Type tuberculosis							
---	-0.02	0.188	0.023	- 0.023	0.142	-0.176	-0.08
P-value	0.904	0.200	0.877	0.877	0.332	0.229	0.587
Acetylators							
---	-0.477**	-0.332*	-0.364*	0.364*	-0.545**	0.571**	0.197
P-value	0.005	0.031	0.018	0.018	0	0	0.202
BMI (kg/m^2)							
----	-0.235	-0.099	0.223	-0.23	-0.103	0.094	0.393**
P-value	0.098	0.443	0.084	0.084	0.422	0.464	0.002
Urea (g/L)							
-----	-0.037	0.186	-0.084	0.084	0.159	-0.175	-0.138
P-value	0.804	0.171	0.539	0.539	0.242	0.197	0.312
Créatinine (g/L)							
-----	0.025	0.038	-0.043	0.043	0.124	-0.108	0.016
P-value	0.868	0.781	0.751	0.751	0.361	0.427	0.905
ALT (IU)							
-----	-0.094	0.137	0.142	-0.142	0.087	-0.087	-0.022
P-value	0.519	0.301	0.284	0.284	0.511	0.511	0.866
AST(IU)							
----	-0.119	-0.057	0.017	-0.017	0.022	-0.002	0.042
P-value	0.412	0.666	0.895	0.895	0.866	0.985	0.75
Hb (g/dl)							
-----	-0.243	0.014	-0.042	0.042	-0.158	0.172	0.13
P-value	0.09	0.915	0.748	0.748	0.224	0.186	0.317
Glucose (g/L)							
----	0.357*	0.107	0.057	-0.057	0.087	-0.102	-0.296*
P-value	0.014	0.419	0.666	0.666	0.511	0.441	0.025
Status HIV							
----	0.150	0.136	0.068	-0.001	0.244	-0.008	-0.182
P-value	0.428	0.472	0.722	0.998	0.193	0.968	0.336

**The correlation is significant at the 0.01 level (bilateral). *The correlation is significant at the 0.05 level (bilateral). b. Calculation impossible because at least one variable is a constant.

Table 3. Pharmacokinetics parameters and HIV.

Parameter	HIV negative	HIV positive	P
	19	11	
$T_{1/2}$ (h)	2.35 ± 0.75	2.47 ± 0.95	0.72
AUC ($mg.l^{-1}.h^{-1}$)	17.90 ± 4.90	21.32 ± 9.23	0.19
Tmax (h)	1.95 ± 0.81	2.18 ± 0.68	0.43
Cmax (mg/l)	3.25 ± 0.75	3.47 ± 0.89	0.48
Clearance ($l.h^{-1}.kg^{-1}$)	0.28 ± 0.08	0.28 ± 0.18	0.96
Vd (l/kg)	0.93 ± 0.31	0.83 ± 0.20	0.33

The differences were not significatives bethween patient's HIV status and pharmacokinetics parameters.

Table 4. Pharmacokinetic parameters and type of acetylators.

Parameter	Genotype slow	Genotype Rapid	P
	17	13	
$T_{1/2}$ (h)	2.66 ± 0,67	2.05 ± 0.88	0.037
AUC (mg.h/L)	22.70 ± 6.28	14.52 ± 4.50	0.0001
Tmax (h)	2.38 ± 0.70	1.58 ± 0.61	0.003
Cmax (mg/l)	3.64 ± 0.63	2.93 ± 0.83	0.012
Clairance ($l.h^{-1}.kg^{-1}$)	0.22 ± 0.05	0.98 ± 0.34	0.001
Vd (l/kg)	0.83 ± 0.20	0.36 ± 0.15	0.135

The differences were not significant between acetylation phenotypes and following pharmacokinetic parameters: $T_{1/2}$, AUC, Tmax, Cmax, and Cl (P 0.037, 0.0001, 0.003, 0.012 and 0.001, respectively), but not significant on Vd.

AUC_{00}, and the slow acetylators are more likely to have a higher INH concentration thus making more side effects exposure (Cho H. et al 2007; Possuelo LG. et al, 2008).

The differences are not significant between the weight and the following pharmacokinetic parameters: $T_{1/2}$, AUC. Our study has highlighted a lack of influence of the weight on the pharmacokinetic parameters. Helen McIlleron et al. (2006) put out the positive impact of weight on increasing the level of exposure (AUC). High weight increases the risk of toxic events appearances. Comparison of pharmacokinetic parameters based on HIV status (Table 5) did not show any significant difference. HIV infection does not change so significantly elements pharmacokinetic analysis contrary to the results of Gurumurthy et al. (2004) who observed a significant decrease in half- life, Tmax, AUC and clearance. The comparison of the two arms (slow acetylators and fast acetylators) office pharmacokinetic characteristics by testing Tau -B and Kendahl shows that there are significant differences between subjects with phenotypes of acetylation on the following pharmacokinetic parameters: $T_{1/2}$, AUC, Tmax, Cmax and Cl (P = 0.037, 0.0001 , 0.003 , 0.012 and 0.001, respectively) but not significant for the Vd. In our study, $T_{1/2}$, AUC, Tmax, Cmax and Cl varied phenotype acetylation, by Vd does not vary against a different phenotype. The slow acetylators subjects tend to metabolize more slowly INH than rapid acetylators. (Cho H. et al 2007), (Possuelo LG. et al, 2008). Their studies showed that NAT -2 acetylator, status of a patient, gender and ethnicity can be considered as significant risk factors for the development of hepatotoxicity.

In summary, the results of the feasibility analysis of plasma drug dosage in Côte d'Ivoire are encouraging, though much remains to be done.

Conclusion

This study provides the originator of data that clinical feasibility of determination of INH is very relevant. The pharmacokinetic parameters were determined by simple mathematical calculations. It shows that patients have rapid acetylators plasma exposure levels INH, and is therefore more likely to make students have serious liver side effects. Given the increase in tuberculosis cases and incidence rate of liver secondary effects and costs associated with hospitalization, it may also be useful to know the state of acetylation of patients before or at the beginning of the initiation treatment against tuberculosis.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Chamorro JG, Castagnino JP, Musella RM, Nogueras M, Aranda (2003). Sex, ethnicity, and slow acetylator profile are the major causes of hepatotoxicity induced by antituberculosis drugs. Gastroenterol Hepatol. 28(2):323-328.

Cho H, Koh W, Ryu Y, Ki C, Nam M, Kim J, Lee S (2007). Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis. Tuberculosis (Edinb) 87 551–556.

Des Prez R, Boone IU (1961). Metabolism of C14-isoniazid in humans. Am. Rev. Respir. Dis. 84:42–51.

Elmendorf DF, Cawthon WU, Muschenheim C, McDermott W (1952). The absorption, distribution, excretion, and short-term toxicity of isonicotinic acid hydrazide (hydrazid) in man. Am. Rev. Tuberc. 65:429–442.

Hoin G (1999). Pharmacocinétique, support de l'enseignement de la pharmacologie générale Associations des enseignants de Pharmacologie des UFR de Pharmacie Ellipse pp. 55-125. http://www.who.int/tb/challenges/gender/page_1/fr/index.html http://www.who.int/tb/challenges/hiv/fr/

Justin J Wilkins, Grant Langdon, Helen McIlleron, Goonaseelan Pillai, Peter J Smith, and Ulrika S H Simonsson (2011). Variability in the population pharmacokinetics of isoniazid in South African tuberculosis patients. Br. J. Clin. Pharmacol. 72:1.

Kergueris MF, Bourin M, Larousse C (1986). Pharmacokinetics of isoniazid: influence of age. Eur. J. Clin. Pharmacol. 30(3):335-340.

Marquet P (2004). Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments Paris: Elsevier, pp. 97-104.

McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P (2006). Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrobiol. Agents Chemother. 50:170–177.

Parkin DP, Vandenplas S, Botha FJ, Vandenplas ML, Seifart HI, van Helden PD, van der walt BJ, Donald PR, van Jaarsveld PP (1997). Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. Am. J. Respir. Crit. Care Me. 155:1717–1722.

Peloquin CA (2002). Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 62:2169–2183.

Possuelo LG, Castelan JA, de Brito TC, Ribeiro AW, Cafrune PI, Picon PD, Santos AR, Teixeira RLF, Gregianini TS, Hutz MH, Rossetti MLR, Zaha A (2008). Association of slow N- acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. Eur. J. Clin. Pharmacol. 64:673–681.

Prema G, Geetha R AK, Hemanth K, Rajasekaran S (2004). Malabsorption of rifampin and isoniazid in HIV-infected patients with and without tuberculosis. Clin. Infect. Dis. 38(2):80-283.

Québec (2009). Center of expertise in environnemental analyse Québec 2009. Protocole pour la validation d'une méthode d'analyse en chimie, DR-12-VMC, Québec, Programme d'accréditation des laboratoires d'analyses, Édition juin.

Rey E, Gendrel D, Treluyer JM, Tran A, Pariente-Khayat A, d'Athis P, Pons G (2001). Isoniazid pharmacokinetics in children according to acetylator phenotype. Fundam. Clin. Pharmacol. 15(5):355-359.

Rey E, Pons G, Crémier O, Vauzelle-Kervroëdan F, Pariente-Khayat A, d'Athis P, Badoual J, Olive G, Gendrel D (1998). Isoniazid dose adjustment in a pediatric population. Ther. Drug Monit. 20(1):50-55.

Rohit B, Indu PK (2012). A sensitive hplc method for determination of Isoniazid in Rat Plasma, Brain, Liver and Kidney. J. Chromat. Separation Techniq. 3:3.

Thee S, Seddon JA, Donald PR, Seifart HI, Werely CJ, Hesseling AC, Rosenkranz B, Roll S, Magdorf K, Schaaf HS(2011). Pharmacokinetics of isoniazid, rifampin, and pyrazinamide in children younger than two years of age with tuberculosis: Evidence for implementation of revised World Health Organization recommendations. Antimicrob Agents Chemother. 55(12):5560-5567.

Vivien JN, Thibier R, Lepeuple A (1973). La pharmacocinétique de l'isoniazide dans la race blanche. Rev Fr Mal Respiratoire. 1:753-773.

World Health Organization (WHO) (2009). Treatment of Tuberculosis: Guidelines. WHO/HTM/TB/2009 420, 4th edn. Geneva: World, Health Organization.

World Health Organization (WHO) (2010). Global Tuberculosis Control: WHO Report 2010. WHO/HTM/TB/2010.7. Geneva: World Health Organization.

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Full Length Research Paper

Ameliorating effect of combination of simvastatin and residronate on glucocorticoid induced osteoporosis model in rats

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Osteoporosis is one of the major problems facing older people of both sexes. This study was undertaken to investigate the synergistic effects of combination of statins and bisphosphonates in a glucocorticoid-induced osteoporosis (GIO) model. Thirty rats were divided into five groups; control group (I), GIO group (II) (given oral prednisolone, pred 30 mg/kg, per 2 days for 6 weeks), group III (treated by pred + risedronate, risedr 1mg/kg), group IV (treated by pred + simvastatin, sim 10 mg/kg) and group V (treated by pred +sim + risedr). Histological study of the femoral neck bone as well as biochemical assessments of serum alkaline phosphatase (ALP), carboxy-terminal collagen crosslinks (CTX), osteoprotegrin (OPG), leptin, Ca and phosphate were performed. The significant decreases in the trabecular bone thickness, area ($p < 0.001$), and the significant increase in bone marrow fat cells area ($p < 0.001$) detected in pred group, were corrected to be nearly similar to control values in groups III, IV and V. Groups that received sim showed better remodeled smoother surfaces bone trabeculae compared with other groups. Combination of sim + risedr shows a significant decrease of bone specific ALP in comparison to pred group ($p < 0.05$). Also, there was significant decrease of CTX in all treated groups in comparison to pred group ($p < 0.05$). On other hand, significant improvements of osteoprotegrin levels were noted in all treated groups ($p < 0.05$), however, there were insignificant changes in leptin levels among groups. In conclusion, addition of statins to bisphosphonate has qualitative rather than quantitative positive effect in experimental osteoporosis which might shorten the duration of therapy and increase efficacy when combined together.

Key words: Statins, bisphosphonates, glucocorticoids, osteoporosis.

INTRODUCTION

Osteoporosis is a common, chronic disease, defined by decreased bone mass and altered microarchitecture,

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resulting in increased bone fragility and susceptibility to fracture (Johnell and Kanis, 2006). It is a common condition, and an economic burden associated with multiple deleterious consequences, including deformity, pain, loss of ambulation, and death. Early prevention and treatment of osteoporosis is very important to avoid these complications. Current drugs for the prevention and treatment of osteoporosis include estrogen, selective estrogen receptor modulators, calcitonin, and bisphosphonates (Cramer et al., 2007), and some of these drugs produce multiple adverse side effects on different tissues (Plotkin et al., 1999).

Despite large problem in the aging society, the discovery of antiresoptive agent is very slow. Also, it was found that women who took statin drugs were less likely to experience a fracture than women who did not take statin drugs (Alexandra et al., 2007).

Despite the development of a number of guidelines for treatment of osteoporosis, management of the condition is not straightforward (Mazziotti et al., 2011; Bultink et al., 2014; Yeh et al., 2014). Several anabolic agents have been investigated in animal models of osteoporosis but it is associated with multiple drawbacks and increase in the risk of fracture may be due to abnormal bone quality (John and Seeman, 2007).

All approved osteoporosis treatments are of the antiresorptive class and it induced a significant decrease in fracture risk, but they do not decrease the risk completely (Alexandra et al., 2007). An increase in bone mineral density did not seem to account for this reduction in fracture risk, so the mechanism by which statins seems to protect against fractures remains unclear (Jadhav and Jain, 2006). So in the present study we try to evaluate role of simvastatin in conjunction of bisphosphonate in osteoporosis model induced by methylprednisolone.

MATERIALS AND METHODS

Drugs

(a). Risedronate (Actonel 5 mg) was dissolved in pathogen free normal saline to make a concentration of 10 mg/ml
(b). Simvastatin (Zocor 40 mg) was dissolved in 4 ml methylcellulose (0.5%) to make a concentration of 10 mg/ml.
(c). Prednisolone

Animals

All procedures were evaluated and approved by the Ethics committee of the University of Mansoura and the study procedures complied with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals. A total of 30 female Sprague–Dawley rats weighing 250 to 275 g were used in this study. During the experiment, animals were housed under controlled environmental conditions and were maintained in plastic cages with free access to food and water and were kept at a constant temperature of 22 ± 1°C with 12 h light/dark cycle for at least 1 week before the experiment.

Animal groups

Rats that survive throughout the study were divided into five groups (each group contained 6 rats):

Group I: Received saline (0.5 ml) served as negative control group.
Group II (pred): Was given oral prednisolone (pred) at 30 mg/kg per 2 days for 6 weeks to produce GIO and serve as positive control (Fujita et al., 2011).
Group III (pred + risedr): Was given daily with pred by above mentioned dose plus receiving risedronate (risedr) orally through NG tube (1mg/kg) (Fujita et al., 2011)
Group IV (pred + sim): Was given daily with pred by above mentioned dose plus receiving simvastatin (sim) orally through NG tube (10 mg/kg) (Wang et al., 2013).
Group V (pred + sim + risedr): Was given daily with pred by above mentioned dose plus receiving sim and risedr as doses mentioned before.

All protocols were approved by our local committee of Animal Care and Use Committee.

Biochemical analysis

At the end of the study, animals were killed under general anesthesia with chloral hydrate (400 mg/kg i.p.) after blood collection by cardiac puncture. Blood was centrifuged and serum stored immediately at -20°C for analysis. Commercially available enzyme linked immunosorbent assay (ELISA) kits for b-ALP (IDS Plc), CTX (Nordic Bioscience Diagnostics, Beijing, China), OPG (IDS Ltd), leptin (Abcam's Leptin Rat ELISA (Enzyme-Linked Immunosorbent Assay) were used to evaluate sera for each animal according to the manufacturer instructions.

Determination of Ca and phosphate (Source: Barnett et al., 1973; Daily and Ertingshausen, 1972)

Histological and electron microscopic study

The hind limbs were disarticulated at the hip. The femurs were carefully dissected and cleared from adjacent muscles. The right femoral neck was processed for preparation of decalcified specimens. First, fixation was done immediately in neutral buffered formaldehyde for 2 days. After fixation, the proximal parts of the right femurs were processed for preparation of decalcified specimens using the chelating agent ethylenediaminetetraacetic acid in the form of its disodium salt (5.5 g ethylenediaminetetraacetic acid in 90 ml distilled water and 10 ml formaldehyde 37 to 40%). Decalcification was carried out for 4 weeks, during which time the decalcifying solution was changed every day (Bancroft and Gamble, 2002). The decalcified specimens were dehydrated and processed to form paraffin blocks. Serial longitudinal sections in the femoral neck, 5 µm thick, parallel to the long axis of the bones, and were prepared. Then, the sections were stained with hematoxylin and eosin stain (H&E) and periodic acid Schiff's stain (PAS) (Bancroft and cook, 1994). The left femoral neck was sectioned with a saw. Specimens were then kept in 2.5%

Table 1. Effect of treatment on Ca, phosphorous and alkaline phosphatase.

Parameter	Ca (mg/dl)	Phosphate (mg/dl)	Alkaline phosphatase (u/ml)
Control	9.63±0.24	8.16±0.22	123.67±2.11
Pred	8.78±0.2	8.55±0.19	130.17±1.4
Pred+risedr	9.42±0.25	7.85±0.2	125.17±2.29[c]
Pred+sim	9.47±0.19	7.88±0.23	127.17±1.47[c]
Pred+sim+risedr	9.73±0.15[b]	7.63±0.19[b]	119.33±1.31[b]

aP < 0.05; when compared to control, bP < 0.05 when compared to positive control; cP < 0.05; when compared to pred + sim + risedr group.

glutaraldehyde after a thorough washing in a buffer solution for a few minutes to remove any debris (Lu et al., 2014). After dehydration and the critical point dryer procedures, tissue samples were mounted on the scanning electron microscope (SEM) stubs with carbon tape and were gold-coated. The bone was examined and photographed with a JEOL JSM 6510 lv SEM (Japan) .

Morphometric study

Digital quantitative assessment of the thickness (in um), the percentage area of the trabecular bone, fat cells and heamopoetic tissue in bone marrow were measured in each histological slice by using an image analyzing software (Image-Pro Plus, version 6.0; Media Cybernetics, Bethesda, Md) (Griffith et al., 2010). The mean of all fields measured from each animal was used for statistical analysis. Histological analysis was carried out by 2 experts (each with more than 10 years experience in histological slides examination) (Griffith et al., 2010).

Statistical analysis

All data are expressed as means ± standard deviation (SD). The significance of differences were assessed by a two-way repeated-measures analysis of variance (ANOVA) followed by Tukey's multiple comparison test. For all other data, comparisons between different treatments were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. In all cases, a probability error of 0.05 was selected as the criterion for statistical significance. Graphs were drawn using GraphPad Prism (version 4.0 for Windows).

RESULTS

Effect of tested drugs on Ca, phosphate and alkaline phosphatase

Combination of sim + risedr increases significantly calcium and decrease significantly phosphate in relation to positive control group ($p < 0.05$) but not exceed physiological limits (Table 1). Also, combination of therapy exerted a significant decrease of bone specific alkaline phosphatse in comparison to positive control group ($p < 0.05$).

Effect of tested drugs on CTX, osteoprotegrin and leptin

There was a significant increase of bone resorption marker (CTX) and significant derangement of osteoprotegrin in positive control group (Table 2). There was significant decrease of CTX in all treated groups in comparison to positive control ($p < 0.05$). On other hand, significant improvements of osteoprotegrin levels were noted in all treated groups ($p < 0.05$). There were insignificant changes in leptin levels among groups (Table 2).

Light microscopic results

In control group, by H&E stain the rat femoral neck was made of cancellous bone made of thick dense bone trabeculae that separated the irregular bone marrow spaces containing hemopoetic cells rather than fatty tissue (Figure 1A). In sections stained with PAS of the control group, intense PAS positive reaction with occasional appearance of cement lines around the osteocytes lacunae were the salient features seen in the bone matrix of the trabeculae (Figure 2A and B).

In the second (pred) group, sections of femoral neck were stained with H&E severe bone resorption in the form of marked thinning and irregularity of the bone trabeculae. The bone marrow spaces were wider and showed marked increase in the fat area compared with the control group (Figure 1B and C). Moreover, the bone matrix exhibited a diffuse decrease in the intensity of PAS stain both/specially in the core of trabeculae and at the resorption bay of the endosteal surface, with complete absence of cement lines. Furthermore there was disarray

Table 2. Effect of treatment on CTX, osteoprotegrin and leptin.

Parameter	CTX (pmol/L)	Osteoprotegrin (pmol/L)	Leptin (ng/ml)
Control	80.17±1.94	45.83±1.87	1.1617± 0.039
Pred	92±2.74[a]	34.67±2.22[a]	1.1967±0 .039
Pred+risedr	79.67±1.9[b]	56±2.52[b,c]	1.1550± 0.022
Pred+sim	79.17±2.32[b]	61±2.35[b]	1.1283± 0.018
Pred+sim+risedr	77.83±2.01[b]	66.67±2.06[b]	1.1267±0 .016

aP < 0.05; when compared to control, bP < 0.05 when compared to positive control; cP < 0.05; when compared to pred + sim + risedr group.

of the endosteal surface of the bone in the form of appearance of resportion bars and irregular spicules (Figure 2C and D).

In the third (pred + risedr) group, by H&E stain, the rat femoral neck showed thicker bone trabeculae with increased bone matrix density compared with the second group. The fat areas in bone marrow spaces are relatively decreased compared with the second group (Figure 1D). The endosteal surface showed a row of activated osteoblasts surrounded by condensed bone matrix. Moreover, there were numerous dark basophilic cement lines around the lacunae of osteocytes in the thickened bone trabeculae (Figure 1E). The one matrix of these trabeculae showed a strong PAS positive reaction (Figure 2E).

In the fourth (pred + sim) group, by H&E stain the rat femoral neck there was homogenously increased bone density observed in most bone trabeculae which appeared thick and dense while few others were thinner and displayed irregular non-homogenous bone density (Figure 1F and G). The bone marrow spaces showed a significant decrease in the fatty tissue area compared with the second group (Figure 1F). Furthermore, the matrix of bone trabeculae exhibited an intense positive PAS reaction (Figure 2F).

In the fifth (pred + sim + risedr) group, by H&E stain the rat femoral neck showed the normal intact bone architecture with almost complete restoration of the bone density and appearance. The bone trabeculae appeared thick and dense together with a considerable decrease of fatty tissue area in bone marrow spaces compared to the second group (Figure 1H). The bone matrix showed a diffuse increase in intensity of PAS stain reaction in the matrix of bone trabeculae compared with the second group (Figure 2G).

Electron microscopic results

By scanning electron microscope (SEM) of the control group, bone trabeculae of the rat femoral neck appeared as thick dense bars with smooth intact surface (Figure 3A). On the other hand, by SEM of the pred per se treated group, there was marked resorption of bone trabeculae of the rat femoral neck which appeared thin, short and atrophied together with multiple erosions and pores of the bone matrix surrounding bone marrow spaces. The bone substance had a decreased density of the bone and marked irregularity of the surface of bone trabeculae (Figure 3B and C) which acquired a smoother surface in the better remodeled bone on accompanying sim either + pred or + risedr in the fourth and fifth, respectively (Figure 3E and F). In addition, by SEM of the third, fourth and fifth groups, there was a relative increase in the density of the bone substance together with a relatively thicker bone trabeculae compared with the second group (Figure 3D to F). On the other hand, the bone marrow spaces appeared relatively narrower in the third group compared with the second group (Figure 3D) and smooth intact surfaces of these trabeculae are seen extending to marrow spaces in the fifth group (Figure 3F).

Statistical analysis of the second group data showed significant decreases in the trabecular bone thickness, area ($p < 0.001$) and hematopoietic tissue area ($p < 0.05$), while it showed a significant increase in bone marrow fat cells area ($p < 0.001$) compared with control group. On the other hand, the other three groups showed significant increases in trabecular thickness area ($p < 0.001$) and hematopoietic tissue area ($p < 0.05$) as well as significant decrease in the bone marrow fat cells area ($p < 0.001$) compared with second group (Figure 4 and Table 3).

DISCUSSION

Osteoporosis is a major public health problem leading to morbidity and mortality in many individuals. Treatment for osteoporosis has generally relied on mechanisms that decrease osteoclastic bone resorption. In the study we evaluated the role statins in treatment of methylprednisolone induced osteoporosis. Effect of statin

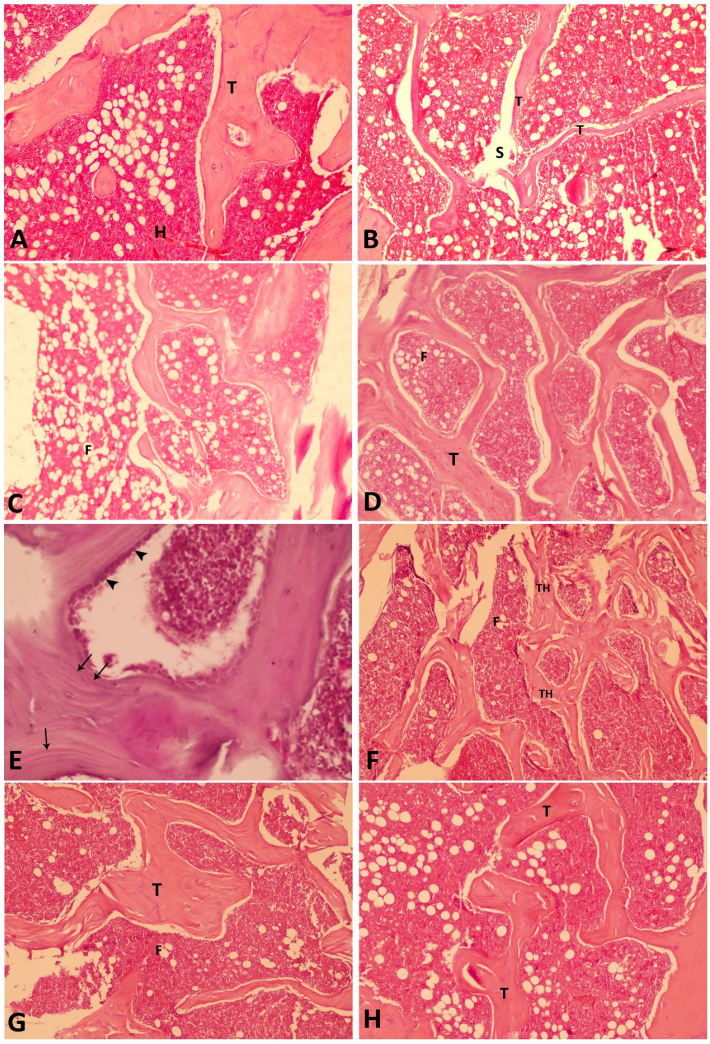

Figure 1. Photomicrographs of sections in the rat femoral neck in all groups stained with H&E. Normal cancellous bone architecture made of thick dense bone trabeculae (T) separated by bone marrow spaces distended with hemopoetic cells (H) and some fatty tissue (arrows) are seen in control group (Figure A). Severe bone resorption in the form of marked thinning of the bone trabeculae (T) with widening of the bone marrow spaces (S) containing a noticeable increased fatty tissue (F) (Figure B and C). In pred + risedr group (Figure D), thicker bone trabeculae (T) with increased bone matrix density compared with the second group is observed with a relatively decreased fatty tissue (F) in bone marrow spaces. Row of activated osteoblasts (arrow heads) at the endosteal surface surrounded by condensed bone matrix containing numerous dark basophilic cement lines (arrows) are also seen in the pred + risedr group (Figure E). Although most of bone trabeculae (T) are thick and dense homogenous in the pred + sim group, some are thinner, irregular and non-homogenous (TH) enclosing bone marrow spaces having minimal fatty tissue (arrow heads) (Figure F and G). Well-formed bone architecture with almost complete restoration of the bone density and appearance are observed in the pred + sim + risedr treated group (Figure H) which exhibits thick dense bone trabeculae (T) surrounding bone marrow spaces distended with hemopoetic cells nearly similar to that of the control group (H&E, Figure A to D, F to H ×100; Figure E ×400).

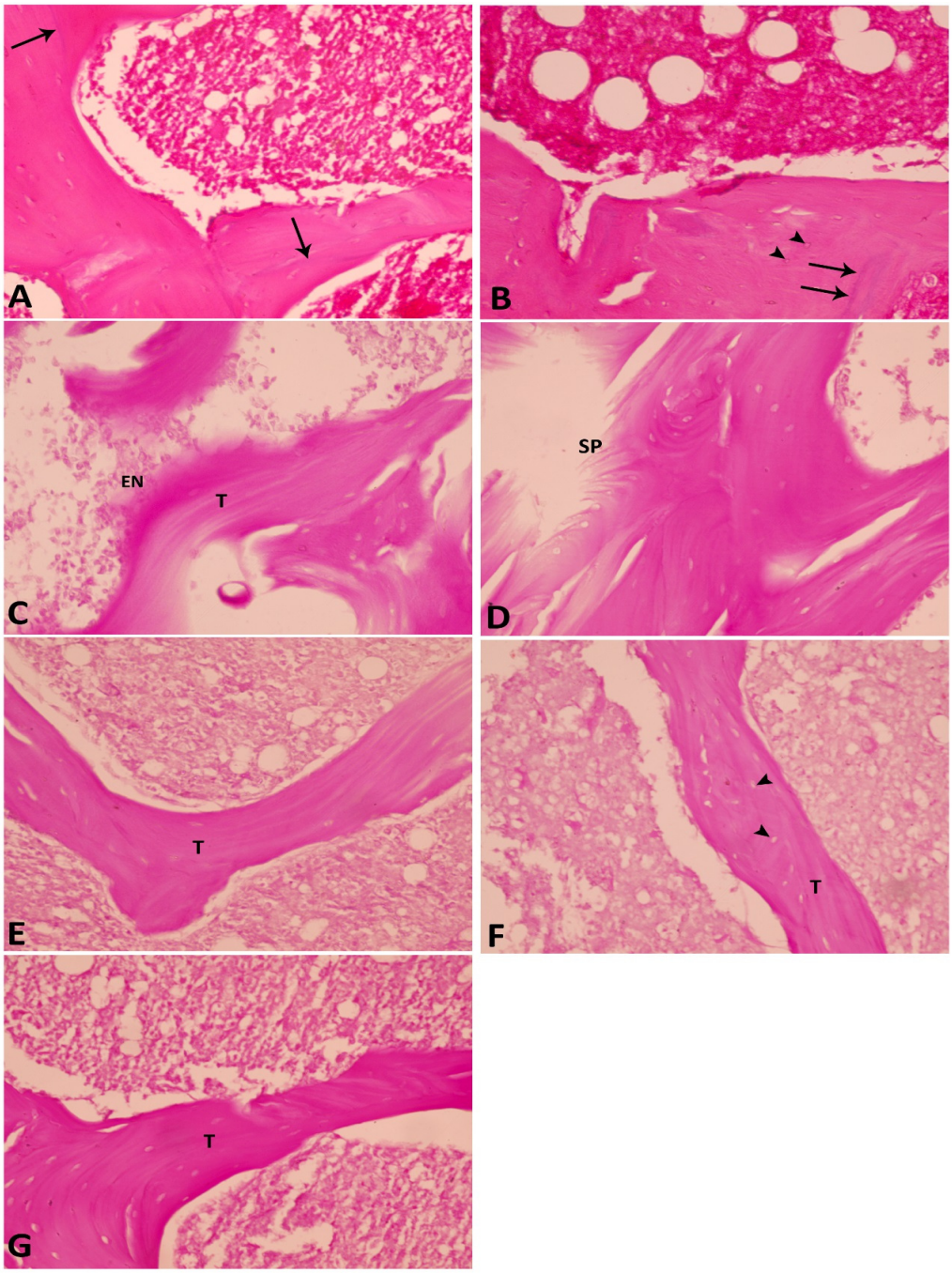

Figure 2. Photomicrographs of sections in the rat femoral neck in all groups stained with PAS. The bone matrix in the trabeculae of the control group (Figure A and B) shows an intense PAS positive reaction with occasional appearance of dark cement lines (arrows) and dispersed bone lacunae (arrow heads). On the other hand, the pred per se treated group (Figure C and D) shows a diffuse decrease in the intensity of PAS stain particularly in the core of trabeculae (T) and at the resorption bay of the endosteal surface (EN) along with appearance of resorbed bars and spicules (SP) and complete absence of cement lines. On the other hand, in pred + risedr treated group (Figure E), strong PAS positive bone matrix in the thickened bone trabeculae (T) is seen. Similarly, in the pred + sim (Figure F) and in the pred + sim + risedr (Figure G) -treated groups, the matrix of bone trabeculae (T) exhibits intense positive PAS reaction surrounding the lacunae of osteocytes (arrow heads) (PAS Figure A to G ×400)

Table 3. Mean and standard deviation of various morphometrical parameters in different groups.

Parameter	Control	Pred	Pred+risedr	Pred+sim	Pred+sim+risedr
Trabecular bone area (%)	28.9±2.8	13.6±4.3**	27.7±3.9$^{\#\#}$	25.8±4.6$^{\#\#}$	30.8±3.5$^{\#\#}$
Bone marrow fat cells area (%)	20.6±3.1	54.9±3.8**	23.6±4.1$^{\#\#}$	26.6±2.9$^{\#\#}$	21.5±2.9$^{\#\#}$
Hematopoietic tissue area (%)	50.5±4.2	31.5±4.5*	48.7±3.4$^{\#}$	47.6±3.7$^{\#}$	47.8±5.1$^{\#}$

*P<0.05 and **P<0.001 compared with control; $^{\#}$P<0.05 and $^{\#\#}$P<0.001 compared with pred per se group.

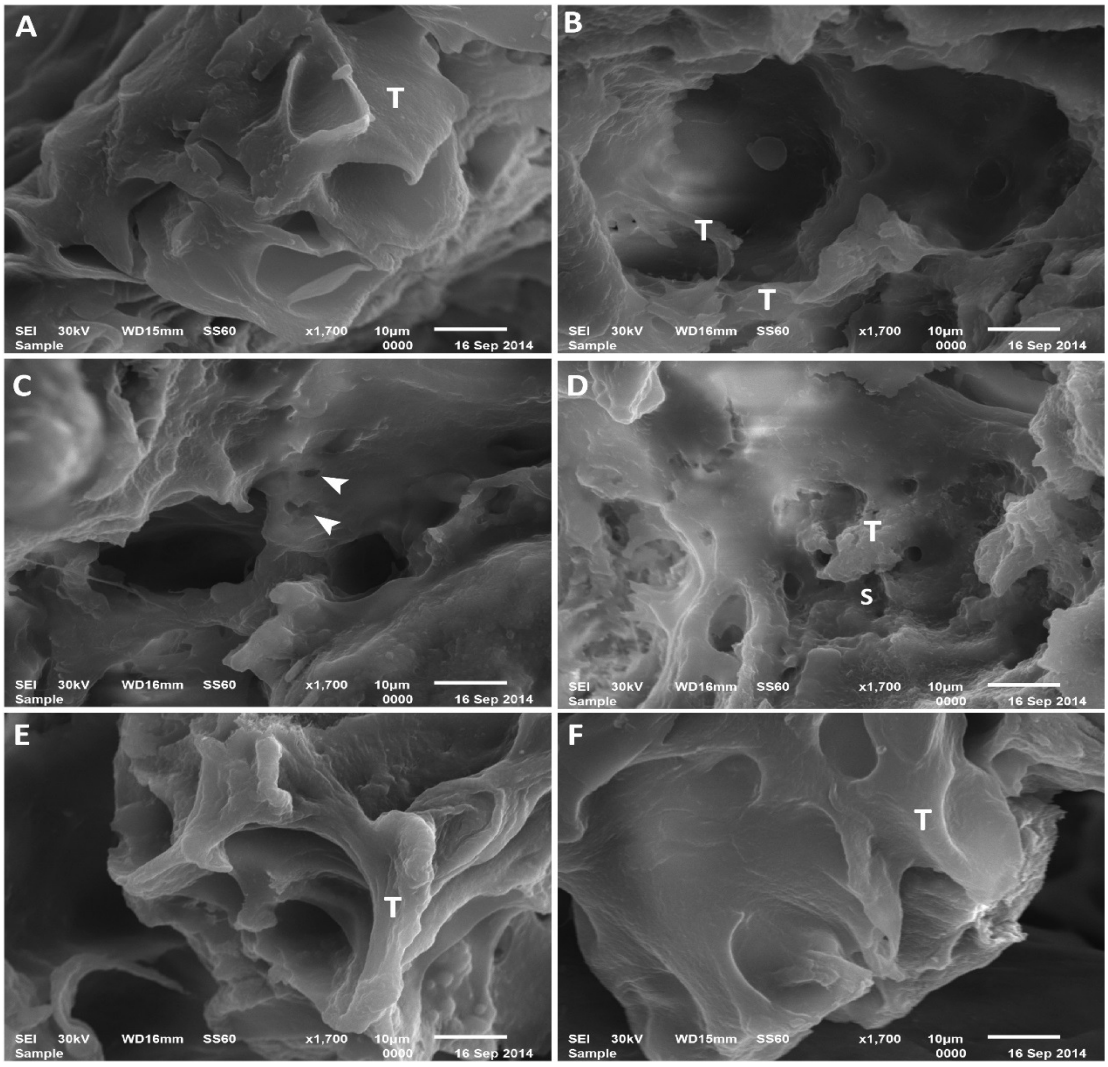

Figure 3. Electron micrographs of sections in rat femoral neck of all groups. The control group displays thick bars of bone trabeculae (T) which have a smooth and intact surface (Figure A). On the other hand, in pred per se treated group (Figure B and C), an obviously decreased density of the bone along with atrophy of its trabeculae (arrows) which appear thin, short and have irregularity in their surface and multiple erosions and pores (arrow heads) in their matrix. However, in the pred + risedr treated group (Figure D), increased density of the bone substance, relatively thicker bone trabeculae (T), but with irregular surface, and relatively narrower bone marrow spaces (S) are observed compared with the second group. Similarly, both pred + sim treated (Figure E) and pred + sim + risedr (Figure F) treated groups exhibit relatively thicker bone trabeculae (arrows), however with a smoother surface and better remodeled, and their bone substance shows a distinct increased in the density compared to the second group (SEM, A to F ×1700)

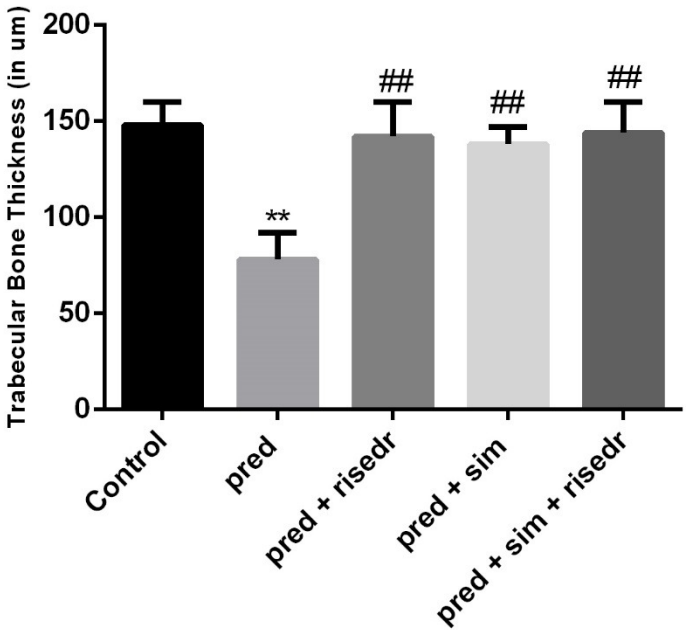

Figure 4. Histogram showing mean ± SD of the trabecular bone thickness (in um) in different groups. **p< 0.001 compared with control
##p<0.001 compared with pred per se treated group.

appear to be mediated through inhibition of bone resorption and stimulation of bone growth, this effect is synergistic when used conjointly with bisphosphonate.

Osteoporosis is a common disease in the elderly population. The progress of this disease results in the reduction of bone mass and can increase the incidence of fractures. Drugs presently used clinically can block the aggravation of this disease. However, these drugs cannot increase the bone mass and may result in certain side effects. Most of the current therapies available for its treatment are limited to the prevention or slowing down of bone loss rather than enhancing bone formation. Recent discovery of statins (HMG-CoA reductase inhibitors) as bone anabolic agents has spurred a great deal of interest among both basic and clinical bone researchers. *In vitro* and some animal studies suggest that statins increase the bone mass by enhancing bone morphogenetic protein-2 (BMP-2)-mediated osteoblast expression.

The ability of bisphosphonates, calcitonin, estrogen and related compounds, vitamin D analogues to increase bone mass is relatively small, certainly not more than 2% per year. It is desirable, therefore, to have a satisfactory and universally acceptable drug that would stimulate new bone formation and correct the disturbance of trabecular microarchitecture, which is a characteristic of established osteoporosis. Although patients using weekly bisphosphonate medication follow their prescribed dosing regimens better than those using daily therapy, overall compliance and persistence rates were suboptimal (McCombs et al., 2004). So the need to add another drug that enhance effect of bisphophosnate and shorten duration of therapy is a good idea and should be evaluated in those categories of patients.

Ruan et al. (2012) said that statins, also known as HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitors, have been widely prescribed for cardiovascular disease (CVD) for decades. Nonetheless, several studies have demonstrated that statins exert bone anabolic effect and may be helpful for the treatment of osteoporosis. Several experiments have analysed the mechanisms of bone anabolism regulated by statins.

Tsartsalis et al. (2012) show statins, osteoporosis and adipogenesis share the same pathway, RANKL/OPG. It would appear that an imbalance in this pathway could be responsible for the manifestation of some metabolic disorders such as diabetes mellitus, atherogenesis, multiple myeloma, osteoporosis. Possibly in the future, drugs which can intervene in this biochemical and pathophysiological cascade, like statins, in a variety of doses, could be used for the management of ectopic ossification syndromes and other bone disorders, even as an additive treatment. Until then, further large longitudinal randomized controlled studies for each statin separately are required to confirm this hypothesis.

Although a limited number of case-control studies suggest that statins may have the potential to reduce the risk of fractures by increasing bone formation, other studies have failed to show a benefit in fracture reduction (Jain, 2006). Randomized, controlled clinical trials are needed to resolve this conflict. One possible reason for the discrepancy in the results of preclinical, as well as clinical studies is the liver-specific nature of statins. Considering their high liver specificity and low oral bioavailability, distribution of statins to the bone microenvironment in optimum concentration is questionable. To unravel their exact mechanism and confirm beneficial action on bone, statins should reach the bone microenvironment in optimum concentration. Dose optimization and use of novel controlled drug delivery systems may help in increasing the bioavailability and distribution of statins to the bone microenvironment. Discovery of bone-specific statins or their bone-targeted delivery offers great potential in the treatment of osteoporosis.

On the other hand, Rejnmark et al. (2004) discussed that sim caused no changes in BMD at the lumbar spine, total hip, femoral neck, or whole body at week 52 or 78. However, a significant increase in BMD was found in response to sim at the forearm. Within the sim group, changes in cholesterol levels did not correlate to BMD changes at any site and conclude that their results do not support a general beneficial effect of sim on bone. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase. Usually, it has been known that they have efficacy on coronary artery diseases and hyperlipidemia (La Rosa and Vupputuri, 1999). Cholesterol synthetic pathway may be important in bone metabolism and that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins may increase bone formation. An experimental observation reported that statins increase bone formation in rodents and that statins have an important role for the cholesterol synthetic pathway in bone formation. This may be via potent bone-forming growth factors, the bone morphogenetic proteins (BMPs). However, other published studies have challenged the effect on fracture risk.

It was found that adipose tissue leptin and OPG expressions are related to osteoporosis in patients with COPD (Pobeha et al., 2011) but in our study no significant changes of leptin was recorded. Some epidemiological studies have suggested that statin use may be associated with increased bone mineral density (BMD) and decreased fracture risk in humans.

Alam et al. (2009) hold that statin/ACS implants show BMP-2 expression and osteoinductive activity that is similar to those of rhBMP-2/ACS implants. Mundy et al. (1999) state that lovastatin and sim increased bone formation when injected subcutaneously over the calvaria of mice and increased cancellous bone volume when orally administered to rats. Thus, in appropriate doses, statins may have therapeutic applications for the treatment of osteoporosis. Another study by Meier et al. (2000) showed that current exposure to statins is associated with a decreased risk of bone fractures in individuals' age 50 years and older. Wang et al. (2000) mentioned the association between statin use by elderly patients and reduction in the risk of hip fracture. Sugiyama et al. (2000) suggest that statins, if they are selectively targeted to bone, have beneficial effects in the treatment of osteoporosis or bone fracture. Garrett et al. (2000) stated that statins increased bone formation and bone mass in rodents, suggesting a potential new action for these compounds, which may be beneficial in patients with established osteoporosis where marked bone loss has occurred. It was found that sim abated oxidative stress, increased NO production, subsequently attenuating osteoporosis. In the *in vitro* studies, the protective effects against H_2O_2-induced cell injury were examined in the MG-63 human osteoblastic cells. It was found that sim ameliorated H_2O_2-induced cell loss and cell apoptosis and increased alkaline phosphatase (ALP) activity in osteoblastic cells (Yin et al., 2012).

In conclusion, the effect of statins on bone mineral density and fracture risk in retrospective studies suggests an exciting new direction for research in bone formation that may lead to advances in the therapy of osteoporosis. Implementation of statins with bisphosphonate may shorten duration of therapy and increase efficacy of drugs.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Alam S, Ueki K, Nakagawa K (2009). Statin-induced bone morphogenetic protein (BMP) 2 expression during bone regeneration: an immunohistochemical study Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiol. Endod. 107(1):22-29.

Alexandra P, Courtney CK, Lisa D, Elaine L, Jonathan DA (2007). Patient adherence to osteoporosis medications. Drugs Aging 24(1):37-55.

Bancroft JD, Cook HC, Turner DR (1994). Manual of histological techniques and their diagnostic application. 2nd ed. USA: Churchill Livingstone.

Bancroft JD, Gamble M (2002). Theory and practice of histological techniques. 5th ed. USA: Churchill Livingstone.

Barnett RN, Skodan SB, Goldberg MH (1973). Performance of kits used for clinical chemistry analysis of calcium in the serum. Am. J. Clin. Pathol. 56 (6):836-845.

Bultink IE, Harvey NC, Lalmohamed A, Cooper C, Lems WF, van Staa TP, de Vries F (2014). Elevated risk of clinical fractures and

associated risk factors in patients with systemic lupus erythematosus versus matched controls: a population-based study in the United Kingdom. Osteoporos Int. 25(4):1275-1283.

Cramer J. A., Gold D. T. & Silverman S. L. et al (2007). Systematic review of persistence and compliance with bisphosphonates for osteoporosis. Osteoporosis Int. 18(8):1023-1031

Daily JA, Ertingshausen G (1972). Direct method for determination of inorganic phosphate in serum with the centrifuge. Clin. Chem. 18:263.

Fujita Y, Watanabe K, Uchikanbori S, Maki K (2011). Effects of risedronate on cortical and trabecular bone of the mandible in glucocorticoid-treated growing rats. Am. J. Orthod Dentofacial Orthop. Mar. 139(3):267-277.

Garrett IR, Esparza J, Chen D, Zhao M, Gutierrez G, Escobedo A, Horn D, Mundy GR (2000): Statins mediate their effects on osteoblasts by inhibition of HMG-CoA reductase and ultimately BMP2. J. Bone Miner Res. 15(Suppl):s225.

Griffith JF, Wang YX, Zhou H, Kwong WH, Wong WT, Sun YL and Ahuja AT (2010). Reduced bone perfusion in osteoporosis: likely causes in an ovariectomy rat model. Radiology 254:739-746.

Jadhav SB1, Jain GK (2006). Statins and osteoporosis: new role for old drugs. J. Pharm. Pharmacol. 58(1):3-18.

Yeh JH, Chen HJ, Chen YK, Chiu HC, Kao CH (2014). Increased risk of osteoporosis in patients with myasthenia gravis: A population-based cohort study neurology. 83:1075-1079.

John MT, Seeman E (2007). New mechanisms and targets in the treatment of bone fragility. Clin. Sci.112:77–91.

Johnell O., Kanis J. A. (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporosis Int. 17(12):1726-1733.

La Rosa JC, He J, Vupputuri S (1999). Effect of statins on risk of coronary disease: a meta- analysis of randomized controlled trials. JAMA 282(24):2340-2346.

Lu H, Yuan G, Yin Z, Dai S, Jia R, Xu J, Lv C (2014). Effects of subchronic exposure to lead acetate and cadmium chloride on rat's bone: Ca and Pi contents, bone density, and histopathological evaluation. Int. J. Clin. Exp. Pathol. 7:640-647.

Mazziotti G, Porcelli T, Mormando M (2011). Vertebral fractures in males with prolactinoma. Endocrine 39(3):288-293.

McCombs JS, Thiebaud P, McLaughlin-Miley C (2004). Compliance with drug therapies for the treatment and prevention of osteoporosis. Maturitas 48:271-287.

Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H (2000). HMG-CoA reductase inhibitors and the risk of fractures. JAMA. 283:3205-3210

Mundy G, Garrett R, Harris S (1999). Stimulation of bone formation in vitro and in rodents by statins. Science 286:1946-1949.

Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T (1999). Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. J. Clin. Invest. 104:1363-1374.

Pobeha P, Ukropec J, Skyba P, Ukropcova B, Joppa P, Kurdiova T, Javorsky M, Klimes I, Tkac I, Gasperikova D, Tkacova R. (2011). Relationship between osteoporosis and adipose tissue leptin and osteoprotegerin in patients with chronic obstructive pulmonary disease. Bone 48(5):1008-1014.

Rejnmark L1, Buus NH, Vestergaard P, Heickendorff L, Andreasen F, Larsen ML, Mosekilde L (2004). Effects of simvastatin on bone turnover and BMD: a 1-year randomized controlled trial in postmenopausal osteopenic women. J Bone Miner Res. 19(5):737-744.

Ruan F, Zheng Q, Wang J (2012) Mechanisms of bone anabolism regulated by statins. Biosci. Rep. 32(6):511-5119.

Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikama S (2000). Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem. Biophys. Res. Commun. 271:688-692.

Tsartsalis AN, Dokos C, Kaiafa GD, et al (2012) Statins, bone formation and osteoporosis: hope or hype? Hormones (Athens) 11:126-139.

Wang L, Duan G, Lu Y, Pang S, Huang X, Jiang Q, Dang N (2013). The Effect of Simvastatin on Glucose Homeostasis in Streptozotocin Induced Type 2 Diabetic Rats. J. Diabetes Res. 5 pp.

Wang PS, Solomon DH, Mogun H, Avorn J (2000). HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA. 283:3211-3216.

Yin H, Shi ZG, Yu YS, Hu J, Wang R, Luan ZP, Guo DH (2012). Protection against osteoporosis by statins is linked to a reduction of oxidative stress and restoration of nitric oxide formation in aged and ovariectomized rats. Eur. J. Pharmacol. 674(2-3):200-206.

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