The combination of atorvastatin and ethanol is not more hepatotoxic to rats than the administration of each drug alone

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Abstract

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Animal studies and premarketing clinical trials have revealed hepatotoxicity of statins, primarily minor elevations in serum alanine aminotransferase levels. The combined chronic use of medicines and eventual ethanol abuse are common and may present a synergistic action regarding liver injury. Our objective was to study the effect of the chronic use of atorvastatin associated with acute ethanol administration on the liver in a rat model. One group of rats was treated daily for 5 days a week for 2 months with 0.8 mg/kg atorvastatin by gavage. At the end of the treatment the livers were perfused with 72 mM ethanol for 60 min. Control groups (at least 4 animals in each group) consisted of a group of 2-month-old male Wistar EPM-1 rats exposed to 10% ethanol (v/v) *ad libitum* replacing water for 2 months, followed by perfusion of the liver with 61 nM atorvastatin for 60 min, and a group of animals without chronic ethanol treatment whose livers were perfused with atorvastatin and/or ethanol. The combination of atorvastatin with ethanol did not increase the release of injury marker enzymes (alanine aminotransferase, aspartate aminotransferase, and lactic dehydrogenase) from the liver and no change in liver function markers (bromosulfophthalein clearance, and oxygen consumption) was observed. Our results suggest that the combination of atorvastatin with ethanol is not more hepatotoxic than the separate use of each substance.

Introduction

Familial hypercholesterolemia is one of the most common genetic disorders with a prevalence of heterozygotes of about 1/500 in most western countries (1). The identification and treatment of patients with hyperlipidemia play an essential role in the primary and secondary prevention of coronary heart disease. Among existing drug therapies, 3-hydroxy-3-methyl-glutaryl co-enzyme and reductase inhibitors, more commonly known as statins, provide a generally well-tolerated and effective option for lowering LDL cholesterol levels and decreasing the likelihood of subsequent coronary heart disease events (2,3).

Statins are used chronically and are the most commercialized class of drugs worldwide (2,3). Sale perspectives for atorvastatin in 2002 were 8.6 billion dollars (4). Atorvastatin differs from other statins in that it has a longer action and presents active metabolites which are biotransformed mainly by CYP3A4 in the liver (5).

Animal studies and premarketing clinical trials point to hepatotoxicity, primarily minor elevations in serum alanine aminotransferase (ALT) levels. For this reason all cholesterol-lowering drugs have labeling that requires the monitoring of liver enzymes (6). The co-factors that modulate statin hepatotoxicity have not been completely elucidated (7,8).

In clinical phase IV studies, 0.5% of the atorvastatin-treated patients showed an increase exceeding three times the normal aminotransferase activity (9). Even with this incidence, discontinuation of treatment is not justified (10). In one premarketing clinical trial with 2045 patients treated with 40 to 80 mg kg^{-1} day^{-1} lovastatin, 3.1% of the patients presented an increase exceeding three times the normal limit of aminotransferase activity. Of these patients, 3 reported alcohol consumption, 2 use of acetaminophen, and 4 of acetaminophen and ethanol (6). In general, patients consuming ethanol were excluded from the clinical trials.

Combined use of medicines and ethanol is common and may present a synergistic action regarding liver injury. There is no systematic study on the effect of chronic use of atorvastatin in the presence of eventual ethanol abuse. Thus, our objective was to conduct an experimental study exploring this possibility.

Material and Methods

Animals and study groups

Male Wistar EPM-1 rats from the Animal House of the Universidade Federal de São Paulo were manipulated according to the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for International Organization of Medical Sciences. Two-month-old rats were chronically treated with ethanol or atorvastatin and, at the end of treatment, their livers were perfused with atorvastatin and/or ethanol. The ethanol group received 10% (v/v) ethanol *ad libitum* 7 days a week for 2 months, this solution substituting water. The atorvastatin group received 0.8 mg/kg of the drug five times a week for 2 months. Control groups consisted of 1) a group of animals exposed to 10% ethanol (v/v) *ad libitum*, substituting water, for 2 months at the end of which period the livers were perfused with 61 nM atorvastatin for 60 min, and 2) a group of animals without chronic treatment whose livers were perfused with atorvastatin and/or ethanol. At the end of treatment each group was divided into 4 subgroups according to the drug used in the liver perfusion experiment: ethanol, atorvastatin, ethanol/atorvastatin combination, and no drug. The initial concentration of 72 mM ethanol added to the perfusion fluid corresponds to alcoholemia that induces an euphoric state in humans. The initial concentration of atorvastatin (kindly donated by Laboratórios Pfizer Ltda., São Paulo, SP, Brazil) in the perfusion fluid was 61 nM (therapeutic blood level in humans).

Liver perfusion experiments

Monovascular rat liver perfusion was performed as previously described (11). Briefly, the rat was perfused with 50 mL Krebs-Henseleit-bicarbonate buffer containing 1.5 g/dL hemoglobin, and 1 g/dL bovine albumin at a 16-mL/min perfusion rate. Samples of perfusate were collected after 30 min for the determination of oxygen consumption and after 60 min for ALT, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) determinations. After

collection of this last sample, the clearance of 11 μmol bromosulfophthalein (BSP) was determined.

Evaluation of liver injury, function, and histology

In order to determine the degree of liver injury the following enzymes were determined in the perfusate: AST, ALT and LDH (reported as $U L^{-1} g$ liver^{-1}) using the ADVIA 1650 Chemistry System (Bayer, Munich, Germany) equipment. Oxygen consumption is reported as mmol L^{-1} g liver^{-1} and BSP clearance as nmol min^{-1} g liver^{-1}. At the end of the experiments, the livers were fixed and stored for subsequent histological analysis with hematoxylin-eosin staining.

Results

Liver injury markers

The livers of animals previously treated with atorvastatin without addition of any drug to the perfusion fluid did not present an increase in ALT release, but when perfused with atorvastatin presented increased ALT activity in the perfusate. In contrast, the group perfused with combined ethanol and atorvastatin did not show an increase in ALT activity (Table 1).

There was no significant increase in AST or LDH activity in any group (Tables 2 and 3).

Table 1. Alanine aminotransferase (ALT) activity (median, N and range) in samples collected after 60-min perfusion.

Pretreatment	ALT (U L^{-1} g liver^{-1})				P
	Addition to perfusion fluid				
	None	Ethanol	Atorvastatin	Ethanol and atorvastatin	
None	0.8 (5) (0.3-0.9)	0.9 (5) (0.3-1.7)	0.5 (4) (0.4-0.6)	1.2 (4) (1.0-1.3)	0.04*
Ethanol	1.5 (4) (0.6-3.2)	1.5 (4) (0.7-2.8)	3.7 (4) (3.3-3.7)	1.2 (5) (0.5-4.0)	
Atorvastatin	0.6 (4) (0.3-1.2)	0.7 (4) (0.5-9.8)	5.0 (6) (1.9-12.4)	1.3 (5) (1.0-1.8)	0.0137[b]
P	0.13	0.46	0.0013[a]	0.70	

*No difference observed in the post-test; [a]difference observed between the samples of the group without previous treatment and those treated with atorvastatin; [b]difference observed between samples of the group perfused without any drug addition and those perfused with atorvastatin [non-parametric analysis (Kruskal-Wallis test and Dunn's post-test)].

Table 2. Aspartate aminotransferase (AST) activity (median, N, and range) in samples collected after 60-min perfusion.

Pretreatment	AST (U L^{-1} g liver^{-1})			
	Addition to perfusion fluid			
	None	Ethanol	Atorvastatin	Ethanol and atorvastatin
None	1.7 (5) (1.5-1.8)	2.8 (5) (1.8-4.5)	1.7 (4) (0.9-2.2)	1.8 (4) (1.2-5.5)
Ethanol	2.6 (4) (1.44-3.70)	4.8 (4) (2.2-8.0)	3.4 (4) (1.8-4.1)	2.3 (5) (1.3-13.9)
Atorvastatin	1.5 (4) (0.9-3.4)	3.4 (4) (0.7-6.6)	4.7 (6) (1.0-13.0)	1.9 (5) (1.5-2.5)
P	0.39	0.30	0.18	0.52

There were no significant differences between groups [non-parametric analysis (Kruskal-Wallis test and Dunn's post-test)].

Evaluation of liver function

There was no difference in oxygen consumption by the liver between the groups studied (Table 4).

The livers of the animals treated with atorvastatin and perfused with ethanol presented a lower BSP clearance rate than the group which received the same treatment and was perfused without any drug addition.

Table 3. Lactate dehydrogenase (LDH) activity (median, N, and range).

Pretreatment	LDH (U L^{-1} g liver^{-1})			
	Addition to perfusion fluid			
	None	Ethanol	Atorvastatin	Ethanol and atorvastatin
None	26.0 (5) (19.2-47.9)	28.2 (5) (20.6-36.8)	24.2 (4) (17.3-30.1)	16.6 (4) (11.6-50.3)
Ethanol	24.0 (4) (18.1-46.3)	35.2 (4) (19.6-46.3)	22.3 (4) (20.3-55.3)	25.2 (5) (7.3-72.7)
Atorvastatin	19.4 (4) (14.7-25.3)	38.6 (4) (16.7-121.4)	41.6 (6) (17.5-125.7)	28.0 (5) (23.9-42.1)
P	0.33	0.98	0.22	0.58

There were no significant differences between groups [non-parametric analysis (Kruskal-Wallis test and Dunn's post-test)].

Table 4. Oxygen consumption (median, N, and range).

Pretreatment	Oxygen consumption (mmol L^{-1} g liver^{-1})			
	Addition to perfusion fluid			
	None	Ethanol	Atorvastatin	Ethanol and atorvastatin
None	0.37 (4) (0.34-0.52)	0.33 (4) (0.21-0.86)	0.45 (4) (0.22-0.55)	0.36 (4) (0.26-0.41)
Ethanol	0.48 (4) (0.28-0.70)	0.54 (4) (0.37-0.62)	0.45 (4) (0.24-0.51)	0.36 (4) (0.25-0.40)
Atorvastatin	0.37 (4) (0.27-0.59)	0.41 (4) (0.12-0.66)	0.34 (4) (0.16-0.40)	0.41 (4) (0.28-0.63)
P	0.67	0.65	0.27	0.63

There were no significant differences between groups [non-parametric analysis (Kruskal-Wallis test and Dunn's post-test)].

Table 5. Bromosulfophthalein (BSP) clearance rate (mean, N, and standard deviation).

Pretreatment	BSP clearance rate (nmol min^{-1} g liver^{-1})				P
	Addition to perfusion fluid				
	None	Ethanol	Atorvastatin	Ethanol and atorvastatin	
None	30.6 (4) (7.5)	26.0 (5) (11.0)	29.7 (4) (1.0)	32.3 (4) (11.5)	
Ethanol	28.7 (4) (10.8)	26.9 (4) (7.7)	19.8 (4) (5.6)	33.4 (5) (3.6)	
Atorvastatin	22.0 (4) (5.1)	11.5 (4) (2.0)	23.4 (5) (6.8)	26.8 (5) (4.0)	0.0027[b]
P	0.33	0.0383[a]	0.07	0.31	

[a]Difference observed between the samples of the group without previous treatment and treated with atorvastatin, both perfused with ethanol. [b]Difference observed between the samples of the group perfused with ethanol and its respective control group (previously treated with atorvastatin without addition of the drug to the perfusion fluid) [parametric analysis (one-way ANOVA and Dunn's post-test)].

However, when the perfusion fluid contained ethanol and atorvastatin this effect was not observed (Table 5).

Histology

Histological examination of the liver was normal except for the presence of faint microvesicular steatosis in the ethanol-treated group. In the atorvastatin-treated group, rare focal lymphocyte and monocyte infiltrates and a slight sinusoid dilatation were observed.

Discussion

Few studies have reported severe atorvastatin hepatotoxicity. Nakad et al. (12) reported a case of atorvastatin-induced acute hepatitis, and Ridruejo and Mandó (13) reported acute cholestatic hepatitis after reinitiating treatment with atorvastatin. Cholestasis and hepatitis induced by statin therapy seem to be rare idiosyncratic reactions (14). Pelli and Setti (15) suggest that atorvastatin may trigger autoimmune hepatitis. Only one case of liver failure was reported by Perger et al. (16) in 2003. The incidence of increased aminotransferase activity in patients who consumed high doses of atorvastatin (80 mg) is 0.5 to 3.3% (17). Although the experience with the safety of statin therapy outside clinical trials which establish ethanol abuse as an exclusion criterion has not been fully reported, it is reasonable to suspect that the incidence of side effects may be higher in clinical situations where patients are not monitored as closely as they are in clinical trials (18).

Moderate ethanol consumption (up to 40 g/day) from 3 to 4 times a week provides a protecting factor against cardiovascular diseases (19). However, the use of combined medications may alter the biotransformation of one of them, increasing hepatotoxicity. Most clinical studies that attempt to prove the safety of statins do not indicate if the patients consumed ethanol (10,20-25). Only Pedersen et al. (26), Newman et al. (27) and Black et al. (9) considered excessive ethanol consumption as an exclusion criterion. Pasternak et al. (8) warned that the concomitant use of ethanol and statins may increase the risk for myopathies.

Smith et al. (28) conducted a retrospective review with 1194 patients who consumed statins. Among those who presented an increase in aminotransferases, only 2 reported ethanol abuse. One of them continued ethanol consumption, with no change in aminotransferase activity, and the other stopped consumption and aminotransferase activities returned to normal.

In the present study, we did not include a group of animals chronically treated with ethanol and atorvastatin. The reason for this was that, although it has been suggested that the chronic and abusive use of alcohol reaches up to 10% of the Brazilian population, 90% of the population as a whole is not considered to suffer from alcoholism.

We observed that livers that had contact with atorvastatin for the first time did not present an increase in ALT activity in the perfusate. However, the group of animals treated and perfused with atorvastatin presented an increase of this enzyme in the perfusate. We did not observe a comparable AST increase, probably because of the different intracellular localization of these enzymes. The chronic use of ethanol combined with acute atorvastatin administration did not cause an increase in injury marker enzymes in the liver (ALT, AST, and LDH, Tables 1, 2, and 3) nor did it cause a change in liver function markers (oxygen consumption and BSP clearance, Tables 4 and 5).

The association of chronic atorvastatin use with acute ethanol abuse also did not cause an increase of ALT, AST, or LDH (Tables 1, 2, and 3) or of oxygen consumption (Table 4). However, there was decrease in the BSP clearance rate in the group where the liver was perfused with ethanol only

(Table 5). No ALT increase occurred in ethanol-treated animals because the dose used is considered low for this experimental animal (29). The fact that atorvastatin promoted an increase in hepatic ALT, but not when combined with ethanol, deserves further study for its elucidation.

Our results suggest that the chronic use of atorvastatin in combination with acute ethanol abuse is not more toxic than the separate use of one or the other substance.

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