Xanthine-oxidase inhibitors and statins in chronic heart failure: Effects on vascular and functional parameters

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BACKGROUND: Increased oxidative stress in heart failure (HF) leads to inflammation and endothelial dysfunction (ED). Both statins and allopurinol have known anti-oxidant properties, but their utility in HF has not been fully assessed.

METHODS: This investigation was a prospective, double-blind, double-dummy study, performed between March 2007 and June 2009. Seventy-four HF patients, with New York Heart Association (NYHA) Class II or III status and left ventricular ejection fraction (LVEF) <40%, were included. Patients received placebo during 4 weeks and were then randomized to receive 4 weeks of either atorvastatin 20 mg/day plus placebo (ATV/PLA group) or atorvastatin 20 mg/day orally plus allopurinol 300 mg/day orally (ATV/ALLO group). Malondialdehyde (MDA), extracellular superoxide dismutase (ecSOD) activity and uric acid (UA) levels, among others, were determined at baseline and after 4 weeks of treatment. ED was assessed by flow-dependent endothelial-mediated vasodilation (FDD), and functional capacity by 6-minute walk test (6MWT).

RESULTS: Thirty-two patients were randomized to ATV/PLA and 38 to ATV/ALLO. Mean age was 59 ± 2 years, 82% were male, and 22% had an ischemic etiology. Hypertension was present in 60% and diabetes in 15% of those studied. No significant differences were observed between baseline measurements and after placebo. After 4 weeks of treatment, both groups showed a significant decrease on MDA (0.9 ± 0.1 to 0.8 ± 0.1 and 1.0 ± 0.5 to 0.9 ± 0.1 μmol/liter, p = 0.88), UA (7.4 ± 0.4 to 6.8 ± 0.3 and 7.2 ± 0.4 to 5.0 ± 0.3 mg/dl, p < 0.01) and FDD (3.9 ± 0.2% to 5.6 ± 0.4% and 4.6 ± 0.3% to 7.1 ± 0.5%, p = 0.07) with increased ecSOD activity (109 ± 11 to 173 ± 13 and 98 ± 10 to 202 ± 16, U/ml/min, p = 0.41) and improved 6MWT (447 ± 18 to 487 ± 19 and 438 ± 17 to 481 ± 21 m, p = 0.83), with all values for ATV/PLA and ATV/ALLO, respectively; p-values are for comparison between groups after treatment.

CONCLUSION: Short-term ATV treatment in heart failure (HF) patients reduces oxidative stress and improves FDD and functional capacity. These beneficial effects are not strengthened by the addition of allopurinol.

Although heart failure (HF) pathogenesis is not fully understood, oxidative stress, inflammation and neurohumoral activity seem to play a role in the development and progression of the disease. Several studies have reported an imbalance between pro- and anti-oxidant activity in HF patients, characterized by a decrease in plasma activity of anti-oxidant enzymes and an increase in reactive oxygen species (ROS) production. This imbalance leads to oxidative modifications of proteins and lipid membranes, reduced...
bioavailability of nitric oxide due to reduced expression of endothelial nitric oxide synthase, and decreased clearance of ROS by the normal anti-oxidant systems, which ultimately contributes to attenuation of endothelial-dependent vasodilation.\(^3\) Interestingly, ROS can act both as triggers or amplifiers of the inflammatory response and they have also been associated with adverse myocardial remodeling, up-regulating expression and activity of matrix metalloproteinases (MMPs).\(^4,5\)

Large clinical trials of statins with coronary artery disease patients have shown a decrease in ventricular remodeling, inhibition of inflammatory cytokine synthesis and ROS production, and an improvement in endothelial function, eliciting considerable interest in the applicability of these results to HF patients.\(^6\) On a similar basis, inhibition of enzymes involved in ROS synthesis, such as xanthine-oxidase (XO), have been considered as a complementary approach for HF management, based on the beneficial effects of allopurinol\(^7\) in small clinical studies showing reduced oxidative stress, mainly due to a reduction in superoxide anion and pro–brain natriuretic peptide (pro-BNP) levels and improved endothelial function. It has not yet been assessed whether these effects are additive in standard-treated HF patients.\(^8\)

The aim of this study was to evaluate the effect of administration of atorvastatin with or without allopurinol in standard HF therapy as it applies to oxidative stress, endothelial function, inflammation, surrogate markers of ventricular remodeling and functional parameters. Our results suggest that addition of statins to standard HF therapy in patients without overt dyslipidemia has a beneficial effect on the aforementioned parameters.

**Methods**

**Patients**

We prospectively included HF patients with New York Heart Association (NYHA) Functional Class (FC) II to IV, who were controlled in a clinical university center. Inclusion criteria were: (a) left ventricular ejection fraction (LVEF) measured by radionuclide-gated pool scan or echocardiogram <40%; (b) standard-of-care pharmacologic treatment, including diuretics, \(\beta\)-blockers, digoxin and angiotensin-converting enzyme inhibitors; (c) stable clinical situation over the proceeding 4 weeks; (d) plasma total cholesterol levels \(\leq 200 \text{ mg/dl}\); and (e) presence of endothelial dysfunction, evidenced as an impaired flow-mediated endothelial-dependent vasodilation (FDD). Exclusion criteria were: (a) acute coronary syndrome in the last 6 months; (b) coronary artery bypass surgery or coronary angioplasty in the last 6 months; (c) uncontrolled arterial hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >90 mm Hg); (d) hypertrophic cardiomyopathy and congenital cardiopathy; (e) use of anti-oxidants, vitamin supplementation, warfarin, allopurinol or statins in the previous 2 months, and (f) presence of other conditions that affect determination of oxidative stress status, such as renal failure (plasma creatinine >2.0 mg/dl), autoimmune diseases, neoplasia, advanced liver or pulmonary disease and acute or chronic inflammation.

This trial was performed between March 2007 and June 2009 and met the Jadad criteria. This randomized, double-blind clinical trial had 5 points (Jadad score) and also had a description of withdrawals and drop-outs. Patients received a placebo for 4 weeks and were then allocated by a computer-generated randomization sequence to receive either 4 weeks of atorvastatin 20 mg/day orally plus identical placebo (ATV + PLA group) or atorvastatin 20 mg/day orally plus identical allopurinol 300 mg/day orally (ATV + ALLO group). There were no modifications in conventional therapy during the study. All patients signed an informed consent form approved by our institutional review board and ethics committee. All patients completed the treatment period. Oxidative stress, ventricular remodeling and inflammation, endothelial function and exercise capacity were determined at baseline and after the therapy. Clinical evaluations were performed at 4 and 8 weeks to identify adverse reactions attributable to the treatment. All patients enrolled completed the study period.

**Assessment of oxidative stress, endothelial function, remodeling, inflammation and exercise capacity**

Plasma malondialdehyde (MDA) levels were determined by examining the content of thiobarbituric acid–reactive substances, as described elsewhere.\(^4\) Standard commercially available MDA (Merck, Darmstadt, Germany) was used. Erythrocyte superoxide dismutase and catalase were determined with standard techniques.\(^7\) For measurement of endothelium-bound superoxide dismutase (ec-SOD), a venous blood sample from the antecubital vein of the non-dominant arm was obtained at baseline. Then, a heparin bolus (5,000 IU) was injected into the brachial artery of the same arm, and blood samples were drawn at fixed intervals from the antecubital vein (1, 3, 5, 7 and 10 minutes after heparin injection). The ecSOD activity was calculated as the area under the curve of the plasmatic SOD activity expressed as units per milliliter of plasma, as described by Landmesser et al.\(^7\) Plasma SOD activity was measured as described by Misra and Fridovic.\(^8\) Surrogate markers for remodeling were evaluated, measuring MMP-2 and MMP-9 activities by gelatin zymography.\(^10,11\) Tissue inhibitor of metalloproteinases 1 (TIMP-1) activity was determined by reverse zymography.\(^12\) Results were expressed as a percentage increase over a reference population of healthy controls. For endothelial function assessment, FDD of the brachial artery was evaluated, as described by Celermajer et al.\(^12\) A 3.0- to 11-MHz lineal ultrasonographic transducer allowed for identification of the percentage change in brachial artery diameter induced by reactive hyperemia after releasing a cuff inflated around the arm to at least 50 mm Hg above systolic pressure for 5 minutes in overnight fasting patients. A change of <8% was considered evidence of endothelial dysfunction.

Vascular cell adhesion molecule-1 was determined by a commercially available enzyme-linked immunoassay (ELISA) test. Serum cholesterol, triglycerides, creatine kinase (CK), serum glutamic oxaloacetic transaminase (sGOT), high-sensitivity C-reactive protein (hsCRP) and uric acid (UA) levels were assessed by routine methods.

Exercise capacity was determined by the 6-minute walk test (6MWTT). For baseline assessment, a practice test was performed for training issues and then a second test was repeated 1 hour later. Variability between both tests should not exceed 15% to be considered valid. In the case of excessive variation, the evaluation was repeated the next day. The highest value among two valid tests was reported as the baseline value. All tests were performed by a trained technician using the standard protocol recommended by the American Thoracic Society.\(^13\)
Statistical analysis

Results are presented as mean ± SEM for continuous variables and as percentage of the total of patients for categorical data. Based on the study by Farquharson and colleagues, we estimated a change in FDD of 9 ± 3% for the associated therapy and a change of 7 ± 3% for statin therapy. For this additional effect, we estimated 36 patients per group to find significant differences with $p < 0.05$ and a power of 80% (paired Student’s $t$-test). Continuous variables were tested for normality using the Kolmogorov–Smirnov test. For comparison between groups, non-parametric tests were used (Mann–Whitney $U$-test and Wilcoxon’s rank sum test for comparison between baseline and placebo group). The chi-square test was used to compare categorical data. For all analysis, $p < 0.05$ (2-tailed) was considered significant. Statistical analyses were performed with SPSS software, version 14.0 (SPSS, Inc., Chicago, IL).

Results

Baseline characteristics

Seventy-four HF patients were included. The mean age was 59 ± 2 years, with 61 (82%) males and 13 (18%) females. HF etiology was ischemic in 16 cases (22%) and idiopathic in 37 (50%) cases. Forty-four (60%) patients had a history of hypertension and 11 (15%) had a history of diabetes mellitus. All patients were clinically stable and the majority were in NYHA FC II and III (100% in ATV+PLA group and 94% in ATV+ALLO group). The mean LVEF was 27 ± 1%. Patients’ characteristics according to allocated group are presented in Table 1. No significant differences in the parameters studied were found during the 4-week placebo run-in. Regarding oxidative stress markers, MDA plasma levels changed from 0.93 ± 0.11 to 0.88 ± 0.10 µmol/liter in the ATV+PLA group at baseline and after placebo, respectively ($p$ = not significant [NS]); meanwhile, in the ATV+ALLO group, levels changed from 1.03 ± 0.11 to 1.03 ± 0.10 µmol/liter ($p$ = NS). In the ATV+PLA group, ecSOD activity was 113 ± 8 and 109 ± 11 U/ml/min at baseline and after placebo, respectively ($p$ = NS), whereas the change was 136 ± 9 to 98 ± 10 U/ml/min in the ATV+ALLO group ($p$ = NS). Last, FDD values changed from 3.9 ± 0.2% to 5.7 ± 0.5% and from 4.6 ± 0.3% to 6.1 ± 0.4% at baseline and after placebo, respectively, in the ATV+PLA and ATV+ALLO groups ($p$ = NS). After randomization, 36 (49%) patients were allocated to the ATV+PLA group and 38 (51%) to the ATV+ALLO group. Patients in the ATV+PLA group showed a higher prevalence of hypertension (72% vs 47%, $p = 0.04$) and a marginally better LVEF (28 ± 1% vs 24 ± 2%, $p = 0.06$).

Effect of atorvastatin and atorvastatin plus allopurinol on oxidative stress

In both treatment groups there was a significant decrease of MDA levels and ecSOD activity when compared with baseline determinations. However, the two treatment strategies were equivalent in their effect on oxidative stress parameters (MDA levels 0.8 ± 0.1 vs 0.9 ± 0.1 µmol/liter [$p = 0.88$] and ecSOD activity 173 ± 13 vs 202 ± 16 U/ml/min [$p = 0.17$] for ATV+PLA vs ATV+ALLO, respectively). As expected, allopurinol use was associated with a significant decrease in UA levels (7.2 ± 0.4 to 5.0 ± 0.3 mg/dl, $p < 0.01$). The use of ATV+PLA was also associated with a significant, albeit discrete, UA-level reduction (7.4 ± 0.4 to 6.8 ± 0.3 mg/dl, $p = 0.02$). The effect of therapy on markers for oxidative stress is shown in Table 2.

Effect of atorvastatin and atorvastatin plus allopurinol on inflammation and remodeling surrogate markers

Both treatments diminished the activity of MMP-9 (0.41 ± 0.03 vs 0.30 ± 0.03 U normalized by standard [$p < 0.01$] and 0.52 ± 0.32 [ $p < 0.01$] for ATV+PLA and ATV+ALLO, respectively), without changes in the activity of the MMP-2, TIMP-1 and vascular cell adhesion molecule (VCAM). No significant differences were observed when comparing the changes observed in the ATV+ALLO vs ATV+PLA groups. In addition, in both treatment groups, there was a decrease in hsCRP, which was significant in the ATV+ALLO group. The data are shown in Table 3.

| Table 1 | Baseline Characteristics in Heart Failure Patients Treated With Atorvastatin (ATV+PLA) and Atorvastatin Plus Allopurinol (ATV+ALLO) |
|-----------------|-----------------|-----------------|-----------------|
| Parameter       | ATV+PLA (n = 36) | ATV+ALLO (n = 38) | p-value          |
| Age, years      | 60 ± 2          | 59 ± 2          | 0.8             |
| Male, n (%)     | 30 (83)         | 31 (82)         | 1.0             |
| Etiology, n (%) |                 |                 |                 |
| Ischemic        | 9 (25)          | 7 (18)          | 0.5             |
| Idiopathic      | 18 (50)         | 19 (50)         |                 |
| Others          | 9 (25)          | 12 (32)         |                 |
| Functional capacity, n (%) |        |                 |                 |
| FC II           | 20 (55)         | 21 (56)         | 0.22            |
| FC III          | 16 (45)         | 15 (38)         |                 |
| FC IV           | 0 (0)           | 2 (6)           |                 |
| Risk factors, n (%) |             |                 |                 |
| Arterial hypertension | 26 (72)       | 18 (47)         | 0.04            |
| Diabetes mellitus | 6 (17)         | 5 (13)          | 0.75            |
| Smoking         | 4 (11)          | 6 (16)          | 0.74            |
| Treatment, n (%) |                 |                 |                 |
| ACE I           | 29 (81)         | 25 (66)         | 0.19            |
| AR A II         | 6 (17)          | 8 (21)          | 0.77            |
| Beta-blockers   | 31 (86)         | 29 (76)         | 0.38            |
| Diuretics       | 27 (75)         | 30 (79)         | 0.79            |
| Spironolactone  | 28 (78)         | 26 (68)         | 0.44            |
| Uric acid (mg/dl) | 7.4 ± 0.4      | 7.2 ± 0.4       | 0.4             |
| (mean ± SEM)    |                 |                 |                 |
| LVEF (%)        | 28 ± 1          | 24 ± 2          | 0.06            |

ARA, angiotensin-receptor antagonist.
Effects of atorvastatin and atorvastatin plus allopurinol in functional capacity, exercise capacity and endothelial function

There was an overall improvement in FC. In the ATV+PLA group, at baseline, 55% of patients were in FC II and 45% in FC III; after treatment, 73% of patients were in FC II and 27% in FC III. On the other hand, in the ATV+ALLO group, at baseline, 56% of patients were in FC II, 38 in FC III and 6% in FC IV; after treatment, 65% of patients were in FC II, 32% in FC III and 3% in FC IV (p-value for comparison between groups was 0.22 at baseline and 0.38 after treatment). In both groups there was an improvement in the 6MWT (from 447±18 to 487±19 m [p = 0.02] and 438±17 to 481±21 m [p = 0.01] for ATV+PLA and ATV+ALLO groups, respectively) and in endothelial function (44% improvement in ATV+PLA and 53% improvement in ATV+ALLO [p < 0.01 in both groups]). Nevertheless, no significant differences were observed between the two treatment strategies in endothelial function or 6MWT. Figure 1 shows individual values of FDD [% of change] at baseline and after intervention for the total group (4.3±0.2% change from baseline vs 6.4±0.4% change from baseline [p < 0.01]), and Figure 2 shows means separated by group. In a subgroup analysis, patients with history of hypertension and ischemic etiology of HF had a tendency toward improvement in FDD after treatment (for patients with history of hypertension: 4.9±1.9 vs 6.7±3.2% for the ATV+PLA and ATV+ALLO groups, respectively [p = 0.05]; for patients with ischemic etiology: 5.3±2.1 vs 9.0±3.3% for the ATV+PLA and ATV+ALLO groups, respectively [p = 0.05]). On the other hand, pro-BNP levels decreased slightly with both treatments: ATV+PLA (2,797±966 to 1,623±275 pg/ml [p = 0.66]) and ATV+ALLO (2,458±425 to 1,640±331 pg/ml [p = 0.20]), without reaching statistical significance when comparing the two groups.

Other results

Total cholesterol levels decreased significantly from 189±7 to 136±6 mg/dl (−28%, p < 0.01) and 186±6 to 144±6 mg/dl (−23%, p < 0.01) in the ATV+PLA and ATV+ALLO groups, respectively. Low-density lipoprotein (LDL) cholesterol also decreased, from 108±5 to 65±5 mg/dl (−40%, p < 0.01) and from 110±6 to 71±4 mg/dl (−35%, p < 0.01) in the ATV+PLA and ATV+ALLO groups, respectively. However, no significant differences in these lipid levels were observed between the two groups. On the other hand, high-density lipoprotein (HDL) cholesterol levels remained unchanged in both experimental groups. Both treatment strategies decreased the levels of total cholesterol, LDL, HDL and triglycerides, as expected (data not shown). No significant adverse effects were reported for either treatment group. Serum GOT and CK activities remained within normal limits.

Discussion

The main finding of the present study is that, in patients with stable HF, the addition of atorvastatin to standard therapy is
associated with a significant decrease in oxidative stress markers, reduced MMP-9 activity, improved endothelial function and improved functional capacity as assessed by 6MWT. Addition of allopurinol to atorvastatin does not further enhance these effects, suggesting that both agents may share a similar mechanism with regard to their beneficial effects described in HF.

In recent years, large-scale clinical trials have demonstrated that administering statins to HF patients already receiving standard therapy is associated with a significant decrease in morbidity and mortality and a marked improvement in ventricular function, regardless of the etiology of the disease. Similarly, we have previously reported a significant decrease in oxidative stress markers and an improvement in endothelial function after 8 weeks of administering atorvastatin 20 mg/day to HF patients.4

The role of XO inhibitors in HF is more controversial. Even when UA level has been proposed as an independent risk factor for mortality in HF, the results are not consistent and its precise role (if any) in HF pathogenesis has not been clarified to date. Several studies have shown the potential benefits of XO inhibitors, such as improved endothelial function and reduced oxidative stress in HF patients. However, this evidence comes principally from animal studies and non-randomized studies with small numbers of patients.

The inactivation of nitric oxide, mediated by anion superoxide, has been shown to be one of the main mechanisms of endothelial dysfunction. Landmesser et al reported that a major source of superoxide is endothelial cell XO (ecXO).7 On the other hand, allopurinol is a substrate and a competitive inhibitor of XO, and its metabolite, oxypurinol, binds in a reversible way. Therefore, a sufficiently high concentration of the drug is required.15 Our results agree with those of George and colleagues, in which urate levels were used as an indicator of the degree of inhibition of XO, showing that, in HF patients, allopurinol 300 mg improved forearm blood flow by 59% compared with placebo, with an additional improvement of 52% when comparing allopurinol 300 mg to allopurinol 600 mg.16

We observed a significant decrease in UA levels in both groups. Of note, the hypouricemic effect of atorvastatin has been described previously among patients with a high risk of cardiovascular disease and in those with coronary artery disease.17 We speculate that the mechanism involved in decreasing UA levels is reduction of ecXO activity related to the decreased activity of rac-1/NADPH oxidase inhibited by statins.7 On the other hand, transcriptional regulation of the expression and activity of XO18 is possible. It is has been demonstrated that inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 and interleukin-6 (IL-6), increase XO expression and activity in endothelial cells and, moreover, different statins are able to reduce expression of these inflammatory cytokines in patients with HF. Therefore, we suggest that atorvastatin may also have reduced ecXO activity—that is, UA levels—in our cohort, by a longer term anti-inflammatory effect. These indirect mechanisms, and the relatively low dose of allopurinol, may explain why the addition of allopurinol did not confer an additive effect on oxidative stress, inflammation, remodeling, endothelial function and functional parameters in our cohort. Moreover, we believe that the concomitant therapy (i.e., beta-adrenergic receptor blockers and angiotensin-converting enzyme inhibitors) could independently decrease ecXO activity.19 In contrast, it is possible that ecXO does not contribute at the biochemical and clinical levels in HF pathophysiology. In fact, in another study we reported a very weak and non-significant correlation between endothelial function (measured by FDD) or 6MWT and ecXO activity in 74 patients with HF.20

The principal limitations of our study are: (a) LV function was not assessed after treatment and we could not provide correlation with the measured outcomes. (b) XO activity was not measured because the assessment of UA levels is an indirect evaluation of the inhibition of XO. (c) Our patients were stable and this could reflect lower oxidative stress and inflammation, which may be the cause of the poor effect of allopurinol in the majority of parameters measured in our study. (d) Covariate hypertension was not investigated in this trial and it would be of interest to determine whether an improvement in
FDD could be seen in the subgroups of hypertension and ischemic patients.

In conclusion, in chronic HF patients with endothelial dysfunction and normal cholesterol plasma levels, treatment with atorvastatin decreases parameters of oxidative stress, inflammation and endothelial function, while improving functional capacity. Addition of the xanthine-oxidase inhibitor, allopurinol, provided no additional cardiovascular benefit.

Disclosure statement

The first two authors (D.G. and H.A.) contributed equally to this work. H.E.V is the recipient of a doctoral fellowship from CONICYT.

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