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Repurposing the atypical antidepressant trazodone for atherosclerotic cardiovascular disease

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Abstract

Background. Current lipid-lowering drugs often leave significant residual risk for adverse outcomes. Identification of previously approved drugs for new indications, drug repurposing, may provide a cost effective alternative to *de novo* drug developing.

Objectives. We combined clinical, transcriptomic, computational, and experimental strategies to explore lipid-lowering and plaque-stabilizing effects of atypical antidepressant trazodone.

Methods. First, a connectivity mapping strategy was used to match rosuvastatin gene expression signature derived from a clinical trial of 85 patients with to the expression patterns of 1,309 different small molecules to discover a similarity between the rosuvastatin and trazodone gene expression signatures. Then, we assessed the lipid-lowering ability of trazodone *in vitro* using HepG2 cells and *in vivo* using molecular imaging of rabbit atherosclerotic lesions. In addition, we analyzed electronic medical records of patients from three large medical centers who had a prescription for trazodone and lipid laboratory measurements available.

Results. Trazodone significantly reduced cholesterol levels in the HepG2 human hepatocyte model, decreased atherosclerotic plaque burden in a rabbit model and lowered low-density lipoprotein (LDL) cholesterol levels in patients.

Conclusion. Our results indicate that trazodone may be a promising candidate for adjunctive lipid lowering therapy. It may provide significant benefits to patients with hyperlipidemia, including lipid level reduction and formation of a more favorable atherosclerotic plaque morphology. Patients diagnosed with major depressive disorder requiring better lipid control would benefit the most from the for adjunctive lipid lowering therapy.

Condensed Abstract

The study combines clinical, transcriptomic, computational, and experimental strategies to demonstrate that the antidepressant trazodone has significant lipid-lowering and plaque-stabilizing effects. First, we demonstrate a similarity between the trazodone and rosuvastatin gene expression signatures. Second, we demonstrate in a hepatocyte model that trazodone decreases cholesterol biosynthesis. Third, we use molecular imaging to demonstrate that trazodone reduces atherosclerotic plaque burden and lowers serum lipid concentrations in a rabbit model. Finally, we analyze electronic medical records of three hospitals and show that trazodone therapy for other indications inadvertently lowers LDL. Our results indicate that trazodone may be a promising candidate for adjunctive lipid lowering therapy.

Introduction

Cardiovascular diseases will remain the most common cause of mortality worldwide through at least 2030. Despite the prevalence of cardiovascular disease, cardiovascular drug development is

underinvested relative to other areas of biomedicine (1). The current front-line pharmacotherapies for atherosclerotic cardiovascular disease (ASCVD) are the statins, which reduce endogenous cholesterol synthesis via the inhibition of the enzyme *3-hydroxy-3-methylglutaryl-CoA reductase* (HMG-CoA reductase) (2). Inhibition of HMG-CoA reductase leads to lower serum concentrations of the atherogenic low-density lipoprotein (LDL) (2-4).

Though statins are effective in reducing adverse cardiovascular outcomes, the need to address substantial residual cardiovascular disease risk among statin-treated individuals has led to the development of new injectable pharmacotherapies such as *proprotein convertase subtilisin/kexin type 9 (*PCSK9*)* or *angiopoietin like 3 (*ANGPTL3*)* modulating drugs (5,6). There is strong evidence that these agents are clinically effective (7), but they are currently not considered cost effective due to their high price tag and the high prevalence of cardiovascular disease secondary to hyperlipidemia–and hence eligibility for lipid-reducing therapy in the US population.(8) Thus, there exists a pressing need for new drugs to prevent ASCVD, especially if new drugs could be discovered more economically or targeted to new patient subsets (9,10).

One technique for addressing high cost of *de novo* drug development is drug repurposing, in which a previously approved compound is applied to a new medical indication (11,12). In this study, we searched for novel compounds with anti-ASCVD action by comparing the *in-vitro* gene expression signatures of cells exposed to drugs with a gene expression signature for high-intensity rosuvastatin therapy that resulted in intracoronary optical coherence tomography-verified plaque stabilization, which was derived from a clinical trial (13). Using this strategy, we found that the atypical antidepressant trazodone, initially approved by the US Food and Drug Administration in 1989, yielded pharmacological effects similar to those induced by statin therapy.

Trazodone is a small molecule compound whose psychiatric effects are thought to function via mixed serotonergic and α-adrenergic action in the central nervous system (14). However, today it is perhaps most commonly prescribed as a temporary, off-label therapy for insomnia (15). The sedative action of trazodone is likely mediated via the histamine H_1 receptor (15). Some recent studies have suggested that a number of psychotropic drugs, including trazodone, may also modulate activity of the enzyme 7-dehydrocholesterol reductase (*DHCR7*) (16). Loss-of-function mutations in both autosomal copies of *DHCR7* dramatically decrease cholesterol levels, leading to the phenotypically severe condition called Smith–Lemli–Oppitz syndrome (SLOS) (17). SLOS can be diagnosed based on elevated serum concentrations of the *DHCR7* substrate 7-dehydrocholesterol (7-DHC); consistent with the notion of a potential cardiovascular benefit, trazodone administration increases levels of circulating 7-DHC in unaffected *DHCR7* mutation carriers or likely carriers (18,19).

Based on these observations, we evaluated trazodone's lipid lowering efficacy in an *in-vitro* model of cholesterol production in a human hepatocyte cell line, and subsequently employed molecular imaging to evaluate its impact on atherosclerotic plaque necrotic core burden in a rabbit model. We also analyzed the electronic medical records (EMR) from three large academic medical centers to assess the

population-level effect of trazodone therapy on LDL cholesterol. Because trazodone has undergone 30 years of post-approval marketing in the United States and abroad, we do not expect that there exist significant, unknown safety concerns related to its effect upon *DHCR7*.

Methods

Drug repurposing. A connectivity mapping strategy was used to match the rosuvastatin gene expression signature to the expression patterns of 1,309 different small molecules (20). We ranked the differential expression of genes from high to low on a per molecule basis, and then computed ordered similarity scores for these 1,309 ranked gene lists. The highest-scoring drugs were those that produced ranked gene lists most similar to the list for rosuvastatin. Score p values (two-side) were obtained empirically by simulating a distribution of 10,000 permutations of null scores from random drug sets, and then fit the observed scores to a Gaussian mixture model with three modes. The resulting p values were then adjusted for multiple hypothesis testing using the Benjamini-Hochberg (BH) procedure to statistically control for false discovery rate.

HepG2 cellular cholesterol production assay. To experimentally verify that trazodone possesses lipid-lowering ability, we first assessed its effects *in vitro* using the HepG2 cell line. The cells were incubated in (1) vehicle solution (DMSO, negative control); (2) 10 μM atorvastatin (positive control); (3) 25 μM trazodone; (4) 50 μM trazodone; or (5) 100 μM trazodone for 24 hours. The effect of each treatment on cellular total cholesterol accumulation was measured in cell lysates as ng of cholesterol/μg of protein with the Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay kit (BioVision, Cat.#: K603-100) (**Supplementary Data. Methods**).

Rabbit model of atherosclerosis. Twenty-five male New Zealand White rabbits weighing from 2.5 to 3.0 kg at maturity were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA). Animals were housed according to NIH and NY State regulations; the experimental protocol was approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai. After two weeks of quarantine, all rabbits were switched to a diet containing 0.3% cholesterol by weight. After one week on hypercholesterolemic diet, rabbits were anesthetized with ketamine and xylazine, intubated, and placed on a ventilator. Under fluoroscopic guidance a 4 French Fogarty catheter (12-040-4F; Edwards Lifesciences, Irvine, CA) was advanced to the level of the diaphragm via an incision in the right femoral artery. The balloon was inflated with 0.75mL of saline solution, and the catheter withdrawn to the level of the aortic bifurcation. This process was repeated five times. The catheter was removed and the arteriotomy site was repaired. The animals were returned to their cage to recover and resume the diet (21).

The animals were randomly assigned to four experimental arms: (1) hypercholesterolemic diet alone (negative control, n=7); (2) hypercholesterolemic diet and 1.0 mg/kg atorvastatin from 3.5 months post-angioplasty until sacrifice (positive control, n=6); (3) hypercholesterolemic diet and 1.0 mg/kg trazodone

from 3.5 months post-angioplasty until sacrifice (low-intensity “preventative” therapy, n=6); (4) hypercholesterolemic diet and 5.0 mg/kg trazodone initiated immediately following angioplasty until sacrifice (high-intensity “recovery” therapy, n=6). Atorvastatin and trazodone dosages were determined by extrapolation from therapeutic human dosages on an equivalent mg/kg basis and were delivered by introducing a needleless 3-cc syringe into the animal’s oral cavity and eliciting the rabbit’s swallowing reflex while simultaneously injecting the syringe’s drug payload. Drugs were administered to each animal once per day for the prescribed period. Over the course of the study, two of the animals assigned to the atorvastatin experimental arm died before the endpoint of 5 months post-angioplasty of apparent liver failure.

Molecular imaging of rabbit atherosclerotic lesions. The lantibiotic peptide duramycin (CAS number 1391-36-2) was conjugated to succinimidyl-6-hydrazinonicotinate acetone hydrazine (S-HYNIC) to provide HYNIC-duramycin, which binds with high specificity to the mammalian cellular membrane phospholipid phosphatidylethanolamine, expressed on the outer leaflet of the cell membrane in cells undergoing apoptosis, a hallmark of the process of atherosclerotic cardiovascular disease. 30 mCi of ^{99m}Tc-pertechnetate in 0.5mL of saline was added to HYNIC-duramycin; ^{99m}Tc-radiolabeled duramycin (^{99m}Tc-Duramycin) was injected via the animal’s marginal ear vein. Four hours later, animals were euthanized and the aortas, blood, urine and tissue samples were collected. The aorta specimens were imaged for 1800 seconds using a dual-head micro-SPECT camera equipped with micro-CT (Triumph SPECT/CT, Trifoil Imaging, Inc; Northridge, California). After the imaging, each aorta was cut into 10–12 pieces ~2 cm in length and activity in each segment was assessed in an automated gamma well counter (PerkinElmer, Gaithersburg, MD, USA) for 600 seconds per segment. Radiation counts were corrected for 99mTc decay from the time of injection, to the injected dose of ^{99m}Tc-duramycin, as well as the weight of the segment. To account for correlations between γ-counts from aortic segments taken from the same animal, data were modeled using a linear mixed effects model (Supplemental Appendix. Methods).

Histopathological examination of rabbit aorta. After gamma counting of the rabbit aorta segments, the vessel segments were fixed by immersion in a solution of 10% formalin for 20 hours, after which they were preserved by immersion in ethanol. These specimens were stained with hematoxylin and eosin and mounted onto slides for light microscopy at New York University School of Medicine’s clinical pathology laboratory using standard procedures.

Electronic Medical Record analysis at three large academic medical centers. We queried de-identified, longitudinal electronic health record systems at three large academic medical centers in the United States: (1) The Mount Sinai Hospital, located in the East Harlem neighborhood of New York, NY; (2) the Columbia University Medical Center, located in the Washington Heights Neighborhood of New York, NY; and (3) the University of California, San Francisco Medical Center, located in the Mission Bay district of San Francisco, CA. Use of de-identified clinical data for retrospective analysis was allowed by the Institutional Review Board at all sites in compliance with all relevant ethical regulations.

We queried for all patients who had a prescription for trazodone, who had serum lipid lab measurements (LDL cholesterol), and for whom we could obtain basic demographic data (e.g., sex, age, and race). Due to differences in data recording and patient populations among the three health systems, the categorical variables included in regression models to control for race and ethnicity differed slightly between datasets (Supplemental Appendix. Methods). To statistically control for the high prevalence of existing anti-lipid therapy, we also determined whether patients had received prescriptions for any commercially available statin (lovastatin, simvastatin, pravastatin, atorvastatin, fluvastatin, rosuvastatin, pitavastatin) at any time point in their health records. We statistically controlled for the effect of statin prescription on lipid values by including a fixed effect term in the regression model. Patients with impossible lipid values (such as negative numbers, likely due to entry error) were manually identified and these observations were removed.

For each patient's lipid tests, lipid values were categorized as having been taken before or after the initiation of trazodone therapy. To appropriately model repeated measurements and within-subject correlation structure in this longitudinal data, for each of the lipid values we fit a linear mixed linear model as described above, with the lipid value as the dependent variable. Age at time of trazodone prescription, gender, race, history of statin therapy, and lab time point (before/after trazodone initiation) were incorporated as fixed effects, and we fit random effects per individual with a random intercept and random slope for laboratory time point slope (which models true baseline increases/decreases in lipid level over time within individuals).

Results

Gene expression signature for high-intensity rosuvastatin therapy. We initiated our search for lipid-reducing drugs based on a gene expression signature associated with high-intensity rosuvastatin therapy, acquired during a clinical trial YELLOW (Reduction in Coronary Yellow Plaque, Lipids and Vascular Inflammation by Aggressive Lipid Lowering) II (13). The trial was designed to detect the effect of high-intensity (40 mg/day) rosuvastatin therapy on atherosclerotic lesion morphology, as assessed via intravascular imaging and concomitant transcriptomic changes in patient peripheral blood mononuclear cells (PBMC). YELLOW II protocol was approved by the institutional review board of the Icahn School of Medicine at Mount Sinai. The obtained gene expression signature contained 117 genes that were differentially expressed between the trial's baseline and follow-up time points (78 up-regulated genes and 39 down-regulated genes). The most highly differentially expressed genes were those classically associated with cholesterol biosynthesis and metabolism, such as the low-density lipoprotein receptor (*LDLR*), squalene epoxidase *(SQLE*), and 24-dehydrocholesterol reductase (*DHCR24*). Notably, the cholesterol biosynthetic enzyme 7-dehydrocholesterol reductase *(DHCR7)* was not contained within this differential expression signature (13).

A search for compounds whose gene expression patterns resemble high-intensity rosuvastatin therapy identified the atypical antidepressant trazodone. Using the connectivity mapping approach described in the Methods section, we ultimately identified nine small molecules whose expression patterns positively

correlated with the rosuvastatin gene signature (i.e., that affected the transcriptome similarly to rosuvastatin) and whose correlations were significant at a false discovery rate of 10%. Of the nine molecules, we chose to proceed with an analysis of trazodone because it is readily available and widely used in clinical practice and in our institution's EMR system.

Trazodone Inhibits Cholesterol Biosynthesis in a Hepatic Cell Line. Treatment of HepG2 cells with either atorvastatin or trazodone inhibited cholesterol levels (**Figure 1**). Specifically, control HepG2 cells (vehicle) had an average of 59.4 ng cholesterol/µg protein lysate, whereas the cholesterol levels from atorvastatin-treated cells were significantly lower (-23.9 ng/µg protein lysate, 95% CI: -38.8 to -8.9, p=0.0046). Similarly, HepG2 cells treated with trazodone at either 50 µM or 100 µM also had lower levels of cholesterol, compared to controls (-35.6 ng/µg protein lysate, 95% CI: -50.6 to -20.7, p<0.0001; and -32.0 ng/µg protein lysate, 95% CI: -47.0 to -17.0, p=0.0003 respectively).

Trazodone Inhibits Necrotic Core Burden in a Rabbit Model of Atherosclerosis. To assess the potential *in vivo* protective plaque-stabilizing effects of trazodone, we performed molecular imaging using duramycin labeled with the Technetium-99m radioisotope. We quantified atherosclerotic lesion burden in the rabbit in two ways. First, we analyzed pixel intensities from single photon emission planar imaging of rabbit aorta. We computationally extracted pixels corresponding to the aortas; the pixel intensities corresponded to the intensity of γ-radiation emitted by the aorta. Representative reconstructed SPECT scans of rabbit aortas are shown in Figure 2. Quantitative analysis of imaging results revealed that rabbits in all three experimental arms (atorvastatin therapy; low-dose, long-duration trazodone therapy; and high-dose, short-duration trazodone therapy) experienced reduced atherosclerotic plaque burden relative to rabbits which did not receive any pharmacotherapy (Figure 3A; Supplemental Appendix. Table 1). Pixel intensities for animals receiving preventative trazodone therapy were 42.7% lower than in control animals (95% CI: -72.1% to -13.2%, p=0.0251), whereas those in the recovery trazodone treatment group were -50.8% lower than control animals (95% CI: -74.9% to -26.8%, p=0.0029). These intensities were similar to those animals who received atorvastatin, which had pixel intensities that were on average 44.6% lower than those of control animals (95% CI: -71.3% to -17.8%, p=0.0118).

Second, we segmented the rabbit aortas into ~2-cm-long pieces and used a well-type γ-radiation counter to quantify the percent of total injected dosage of ^{99m}Tc-duramycin complex that was retained in the aorta, normalized against the mass of the aorta segment (^{99m}Tc-duramycin per gram). Analysis of the percentage of retained total injected dosage of ^{99m}Tc-duramycin complex revealed findings similar to those obtained by SPECT: rabbits in all three experimental arms retained less ^{99m}Tc-Duramycin (Figure 3B; Supplemental Appendix. Table 2). Specifically, animals receiving the high-dose, short-duration trazodone therapy had 37.2% less retained ^{99m}Tc-duramycin per gram than control animals (95% CI: -63.9% to -17.2%, p=0.0020). Animals receiving the low-dose, long-duration trazodone therapy had 52.1% less retained ^{99m}Tc-duramycin per gram (95% CI: -83.0% to -29.0%, p=0.0002). These values were similar to those in animals that received atorvastatin, which had 41.1% less retained ^{99m}Tc-duramycin per gram (95 CI: -70.1 to -19.4, p=0.0017).

Histopathological examination of rabbit aorta. We next examined pathological specimens of rabbit aorta with light microscopy. Representative findings are shown in Figure 4. Rabbits fed a hypercholesterolemic diet without pharmacological intervention exhibited extensive atherosclerosis, primarily with fibroatheromatous plaques with large necrotic cores, cholesterol clefts, and extensive inflammation with numerous macrophages. The plaques of animals treated with atorvastatin were predominantly fibrocalcific with small residual necrotic core and significantly decreased number of macrophages. Preventative-dose trazodone rabbits had predominantly fibrous plaques, with small necrotic cores and markedly decreased macrophages. Similarly, recovery-dose trazodone rabbits had predominantly fibrous plaques with residual and decreased macrophages.

Trazodone decreases LDL cholesterol, as demonstrated in three large urban health systems. To assess the potential lipid-lowering effect of trazodone in humans, we examined the EMR for 13,026 patients who received prescriptions for trazodone in three large urban healthcare systems (Table 1). Specifically, we considered only patients with at least one LDL cholesterol result and who had at least one prescription for trazodone of any dosage. Our effect estimates are likely strongly biased toward the null because trazodone is more frequently prescribed for short-term insomnia, and we could not reliably assess the duration of active therapy.

Using these criteria to analyze low-density lipoprotein, we identified 2,698 patients at the Mount Sinai Hospital in New York City (MSH), 5,289 at Columbia University Medical Center in New York City (CUMC), and 2,852 at the University of California, San Francisco Medical Center in the Mission Bay district of San Francisco (UCSF). At MSH, patients who took trazodone had LDL cholesterol levels 3.73 mg/dL lower in lipid panels conducted the year following the initiation of trazodone therapy ($p=0.0004$). At CUMC and UCSF, the reductions were 1.06 mg/dL ($p<0.0001$) and 0.67 mg/dL ($p=0.013$), respectively.

Analysis of Rabbit Serum Lipid Levels. We analyzed serum LDL cholesterol levels for a subset of rabbits assigned to either the hypercholesterolemic diet plus preventative-dose trazodone therapy (n=5) or the hypercholesterolemic diet with no drug intervention (n=3). Mean LDL cholesterol level for the animals given the hypercholesterolemic diet alone was 658 mg/dL, vs. 549 mg/dL in animals given the hypercholesterolemic diet plus preventative trazodone. This difference of 109 mg/dL was statistically significant ($p=0.0357$, Wilcoxon signed-rank test).

Discussion

Using a multifaceted approach, we have established several major findings that together provide strong evidence for the use of the atypical antidepressant trazodone as a lipid-lowering therapy capable of necrotic core burden reduction and plaque stabilization (Figure 6). This study offers a unique approach that extends from bedside to bench and back and is representative of translational research. First, the antidepressant trazodone induced changes in gene expression similar to those observed in human patients undergoing 8–12 weeks of rosuvastatin therapy. In fact, from a compendium of 1,309 small

molecules, trazodone was the second most similar to rosuvastatin. This similarity was even higher than those of all of the statins included in the set of 1,309 tested compounds.

Second, trazodone significantly reduced cholesterol levels in the HepG2 human hepatocyte model. In mammals, cholesterol biosynthesis is a highly evolutionarily conserved process that occurs primarily in the cytosol and endoplasmic reticulum of hepatocytes. Our observation that trazodone reduces cholesterol levels in a hepatocyte model is a significant finding, since it implies that the transcriptomic similarity we observed between rosuvastatin and trazodone causes them to exert similar effects on cholesterol biosynthesis. This finding also provides evidence that the lipid-lowering effects of trazodone are not somehow mediated indirectly via the psychiatric effects: HepG2 cells, as hepatocytes, lack most of the important distinguishing characteristics of cells found in the central nervous system.

Third, using a rabbit model of atherosclerotic disease (where the endothelial layer of rabbit aorta is injured with a balloon catheter and then the animal is subsequently fed a pro-atherosclerotic diet), we demonstrated that there were significant decreases in pathologically defined atherosclerotic lesions in animals fed trazodone relative to control animals. It is important to note that the signal from the radionuclide imaging employed in this study (^{99m}Tc-HYNIC-Duramycin) is specific for detection of cell death by virtue of its ability to bind the cellular phospholipid membrane component phosphatidylethanolamine. Inefficient efferocytosis indicates that cells dying by apoptosis are not promptly phagocytized, allowing noxious intracellular substances to leak into surrounding tissues, increasing the inflammatory response, contributing to the formation of a necrotic core. Thus, ^{99m}Tc-duramycin signal intensity provides quantitative insight into atherosclerotic plaque inflammation. Since histopathological examination revealed that trazodone decreased necrotic core formation in a fashion similar to atorvastatin, we consider this to be an orthogonal validation of the effectiveness of trazodone.

Finally, we have evidence for the atheroprotective effect of trazodone from more speculative findings obtained via analysis of EMRs in three different health systems. Our EMR findings are perhaps more equivocal than the anatomic studies since they show only a modest effect of trazodone on lipids. However, this is likely due to the fact that the primary use of trazodone at present is as a sleep aid; for this indication, the drug is often used only for a short time, so we would expect that its observed effects on lipids in an observational study would be relatively small. The observed heterogeneity of the effects of trazodone, which were much stronger at Mount Sinai than at either Columbia University Medical Center or at the University of California Health System, could be explained in any of several ways. Retrospective EMR analyses may be prone to subtle forms of bias, so it is quite possible that there were unexpected and unexplored confounders that could account for this heterogeneity. Ultimately, understanding the anti-lipid action of trazodone in humans will require prospective randomized clinical trials. Consistent with our clinical findings, however, in our animal studies we observed a significant difference (109 mg/dL) in LDL concentration between trazodone-treated and control rabbits.

Even if trazodone were conclusively demonstrated to be an effective anti-lipid and cardioprotective therapy, there could still be significant obstacles to its use in the cardiovascular disease setting. First,

trazodone's sedative action and its primary indication as an antidepressant are widely acknowledged. Although there is emerging epidemiological evidence for a significant link between psychiatric diseases such as depression and chronic cardiovascular disease (22,23), antidepressant therapy is not appropriate for all patients. Second, the sedative effect, although diminishing over time, would likely prove to be a significant factor in the clinical usage of trazodone. For example, patients may only be able to take trazodone before bed for the first few weeks to months because it causes drowsiness and somnolence. Finally, many psychotropic drugs can produce a range of potential cardiovascular adverse effects, including orthostatic hypotension, hypertension, tachycardia, or alterations in cardiac conduction and promotion of a proarrhythmic state (22,23). However, relative to other classes of antidepressants, such as the selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors, and older tricyclics, trazodone and other atypical antidepressants are less likely to cause severe cardiovascular side effects. Of note, isolated studies have reported that trazodone may, in rare circumstances, lead to effects such as QT prolongation (24).

Conclusions

In summary, it is unlikely that trazodone will prove to be a "silver bullet" against atherosclerotic cardiovascular disease. However, it may yet play an important role as another possible tool, at least as a booster therapy, to address the residual remaining risk for individuals whose cholesterol levels are not adequately controlled with statin therapy alone. From the standpoint of cost, trazodone is an especially attractive candidate because it is readily available in an inexpensive generic form. In addition, it might be especially beneficial in patients with hyperlipidemia comorbid with conditions trazodone is already used to treat, such as depression or insomnia. As noted, however, definitive results in humans will require randomized controlled trials. Nonetheless, at this point our data suggest that trazodone may yield significant benefits in patients with hyperlipidemia, including lipid level reduction and formation of a more favorable atherosclerotic plaque morphology. It may also be useful for patients already diagnosed with major depressive disorder who require better lipid control.

Limitations

The main limitation of the study is the retrospective nature of the EMR analysis of lipid-lowering effect of trazodone in patients. The findings are potentially confounded and the differences, while formally significant are rather small. A fully powered randomized clinical trial is needed to provide strong evidence for the association between trazodone and LDL-C. The lack of randomization in the YELLOW II study represents another limitation of the analysis.

Perspectives

Competency in Medical Knowledge: Statins are effective in reducing adverse cardiovascular outcomes; however, there is a need to address substantial residual cardiovascular disease risk among statin-treated individuals whose cholesterol levels are not adequately controlled with statin therapy alone. Atypical

antidepressant trazodone yielded pharmacological effects similar to those induced by statin therapy. Trazodone may be a promising candidate for adjunctive lipid lowering therapy, especially in patients diagnosed with major depressive disorder requiring better lipid control.

Translational Outlook: Trazodone may yield significant benefits in patients with hyperlipidemia, including lipid level reduction and formation of a more favorable atherosclerotic plaque morphology. Understanding the anti-lipid action of trazodone in humans will require prospective randomized clinical trials.

Abbreviations

3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase)

7-dehydrocholesterol reductase (*DHCR7*)

7-dehydrocholesterol (*7-DHC*)

Atherosclerotic cardiovascular disease (ASCVD)

Low-density lipoprotein (LDL)

Single photon emission computed tomography (SPECT)

Declarations

Dr. Baber has received speaker honoraria from AstraZeneca and Boston Scientific and honoraria from Amgen; Dr. Dudley and Dr. Johnson are employees of and hold equity in Tempus Labs (Chicago, IL); Dr. Pak and Dr. Gray are employees of Molecular Targeting Technologies (West Chester, PA, USA), which is the vendor that provided the kit formulation of duramycin conjugated to succinimidyl-6-hydrazinonicotinate acetone hydrazine (S-HYNIC) used to perform SPECT molecular imaging of rabbit atherosclerotic plaques at the Icahn School of Medicine at Mount Sinai by approved investigators. The involvement of Drs. Pak and Gray consisted of design and validation of the imaging molecule kit and of ensuring accuracy in the description of the molecule in the methods section of the manuscript. The other authors have nothing to disclose.

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References

1. Fordyce CB, Roe MT, Ahmad T et al. Cardiovascular drug development: is it dead or just hibernating? J Am Coll Cardiol 2015;65:1567–82.
2. Sirtori CR. The pharmacology of statins. Pharmacol Res 2014;88:3–11.

3. Cholesterol Treatment Trialists C, Baigent C, Blackwell L et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 2010;376:1670–81.
4. Jarcho JA, Keaney JF, Jr. Proof That Lower Is Better–LDL Cholesterol and IMPROVE-IT. N Engl J Med 2015;372:2448–50.
5. Olkkonen VM, Sinisalo J, Jauhiainen M. New medications targeting triglyceride-rich lipoproteins: Can inhibition of ANGPTL3 or apoC-III reduce the residual cardiovascular risk? Atherosclerosis 2018;272:27–32.
6. van der Laan SW, Harshfield EL, Hemerich D, Stacey D, Wood AM, Asselbergs FW. From lipid locus to drug target through human genomics. Cardiovasc Res 2018.
7. Ghadban R, Enezate T, Omran J, Almourani R, Singla A, Balla S. Clinical outcomes of PCSK9Is: a meta-analysis of randomized clinical trials. Cardiovasc Diagn Ther 2017;7:598–606.
8. Arrieta A, Hong JC, Khera R, Virani SS, Krumholz HM, Nasir K. Updated Cost-effectiveness Assessments of PCSK9 Inhibitors From the Perspectives of the Health System and Private Payers: Insights Derived From the FOURIER Trial. JAMA Cardiol 2017;2:1369–1374.
9. Johnson KW, Torres Soto J, Glicksberg BS et al. Artificial Intelligence in Cardiology. J Am Coll Cardiol 2018;71:2668–2679.
10. Johnson KW, Shameer K, Glicksberg BS et al. Enabling Precision Cardiology Through Multiscale Biology and Systems Medicine. JACC Basic Transl Sci 2017;2:311–327.
11. Hodos RA, Kidd BA, Shameer K, Readhead BP, Dudley JT. In silico methods for drug repurposing and pharmacology. Wiley Interdiscip Rev Syst Biol Med 2016;8:186–210.
12. Shameer K, Glicksberg BS, Hodos R et al. Systematic analyses of drugs and disease indications in RepurposeDB reveal pharmacological, biological and epidemiological factors influencing drug repositioning. Brief Bioinform 2017.
13. Kini AS, Vengrenyuk Y, Shameer K et al. Intracoronary Imaging, Cholesterol Efflux, and Transcriptomes After Intensive Statin Treatment: The YELLOW II Study. J Am Coll Cardiol 2017;69:628–640.
14. Mittur A. Trazodone: properties and utility in multiple disorders. Expert Rev Clin Pharmacol 2011;4:181–96.
15. Atkin T, Comai S, Gobbi G. Drugs for Insomnia beyond Benzodiazepines: Pharmacology, Clinical Applications, and Discovery. Pharmacol Rev 2018;70:197–245.
16. Kim HY, Korade Z, Tallman KA et al. Inhibitors of 7-Dehydrocholesterol Reductase: Screening of a Collection of Pharmacologically Active Compounds in Neuro2a Cells. Chem Res Toxicol 2016;29:892–900.
17. Boland MR, Tatonetti NP. Investigation of 7-dehydrocholesterol reductase pathway to elucidate off-target prenatal effects of pharmaceuticals: a systematic review. Pharmacogenomics J 2016;16:411–29.

18. Hall P, Michels V, Gavrilov D et al. Aripiprazole and trazodone cause elevations of 7-dehydrocholesterol in the absence of Smith-Lemli-Opitz Syndrome. Mol Genet Metab 2013;110:176–8.
19. Korade Z, Genaro-Mattos TC, Tallman KA et al. Vulnerability of DHCR7(+/-) mutation carriers to aripiprazole and trazodone exposure. J Lipid Res 2017;58:2139–2146.
20. Lamb J, Crawford ED, Peck D et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 2006;313:1929–35.
21. Giannarelli C, Cimmino G, Connolly TM et al. Synergistic effect of liver X receptor activation and simvastatin on plaque regression and stabilization: an magnetic resonance imaging study in a model of advanced atherosclerosis. Eur Heart J 2012;33:264–73.
22. Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. Eur Heart J 2014;35:1365–72.
23. Pina IL, Di Palo KE, Ventura HO. Psychopharmacology and Cardiovascular Disease. J Am Coll Cardiol 2018;71:2346–2359.
24. Mazur A, Strasberg B, Kusniec J, Sclarovsky S. QT prolongation and polymorphous ventricular tachycardia associated with trasodone-amiodarone combination. Int J Cardiol 1995;52:27–9.

Tables

Table 1: Baseline demographics and clinical characteristics for patients receiving trazodone and lipid tests at three centers

- *1. Mount Sinai Hospital, New York, NY*
- *2. University of California, San Francisco, San Francisco, CA*
- *3. Columbia University Medical Center, New York, NY*

Figures

	MSH[1]	UCSF[2]	CUMC[3]
Patient and Laboratory Counts			
With ≥1 trazodone prescription &LDL-C Measurement, n (%)	2698	2,852	11,479
LDL-C Measurements, n	10,524	48,814	95,356
Mean LDL-C (mg/dL) ± SD	92.3±37.80	100.01±36.04	100.42±47.49
Age			
Mean Age (years) ± SD	57.87±17.39	61.14±15.88	57.02±17.15
Gender			
Male Gender, n (% of total)	1361 (50.44%)	1160 (40.67%)	5,002 (43.58%)
Female Gender, n (% of total)	1337 (49.56%)	1692 (59.33%)	6,477 (56.42%)
Race/Ethnicity			
Caucasian/White, n (% of total)	407 (15.09%)	1471 (51.58%)	2,318 (20.19%)
African American or Black, n (% of total)	453 (16.79%)	308 (10.80%)	924 (8.05%)
Hispanic/Latino, n (% of total)	20 (0.74%)	-	2,257 (19.66%)
Asian, n (% of total)	547 (20.27%)	565 (19.81%)	70 (0.61%)
Other, unknown, or multi-racial, n (% of total)	1271 (47.11%)	508 (17.81%)	5,910 (51.49%)
Statin Therapy			
Receiving statin therapy, n (% of total)	1158 (42.92%)	1176 (41.23%)	5,999 (52.2%)

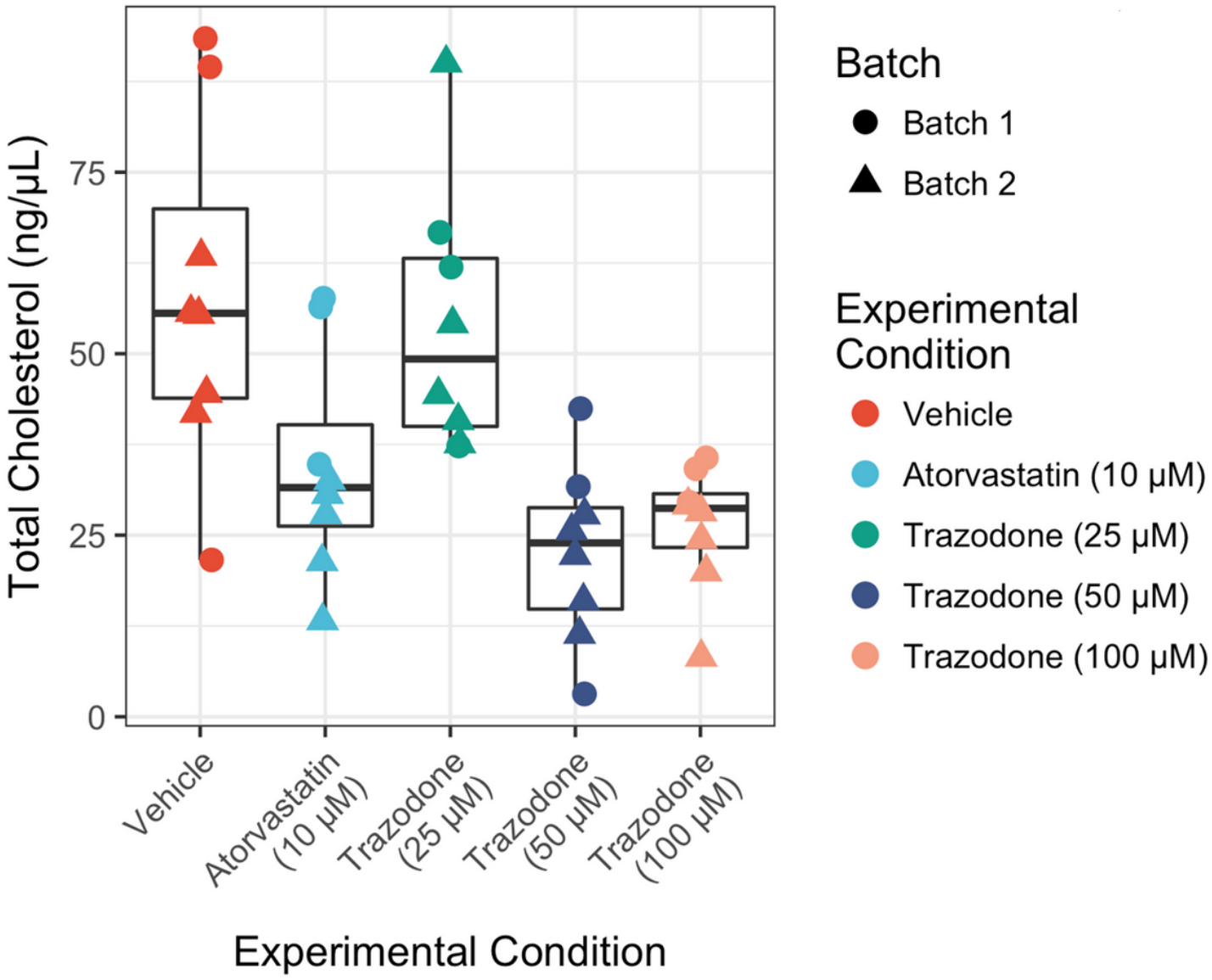

Figure 1

HepG2 cells treated with 10 µM atorvastatin, 50 µM trazodone, or 100 µM trazodone have significantly lower total cholesterol levels than control HepG2 cells treated with vehicle (DMSO) (p=0.0046, p=<0.0001, and p=0.0003 respectively). HepG2 cells treated with 25 µM trazodone did not differ significantly from control.

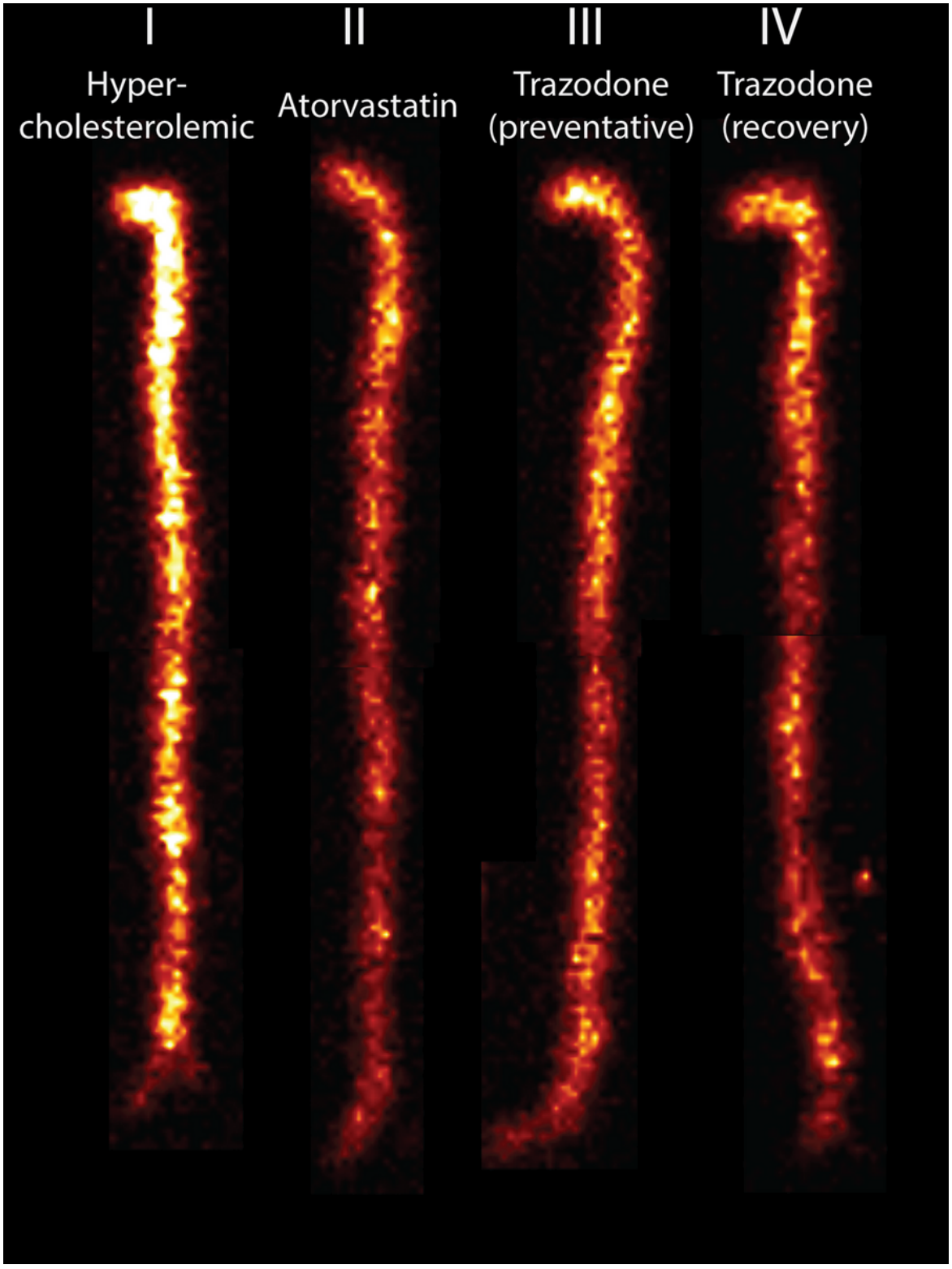

Figure 2

Four reconstructed representative single photon emission planar aorta scans. Aorta I: hypercholesterolemic diet. Aorta II: hypercholesterolemic diet with atorvastatin. Aorta III: hypercholesterolemic diet with high-intensity, short-duration (recovery) trazodone therapy. Aorta IV: hypercholesterolemic diet with low-intensity, long-duration (preventative) trazodone therapy.

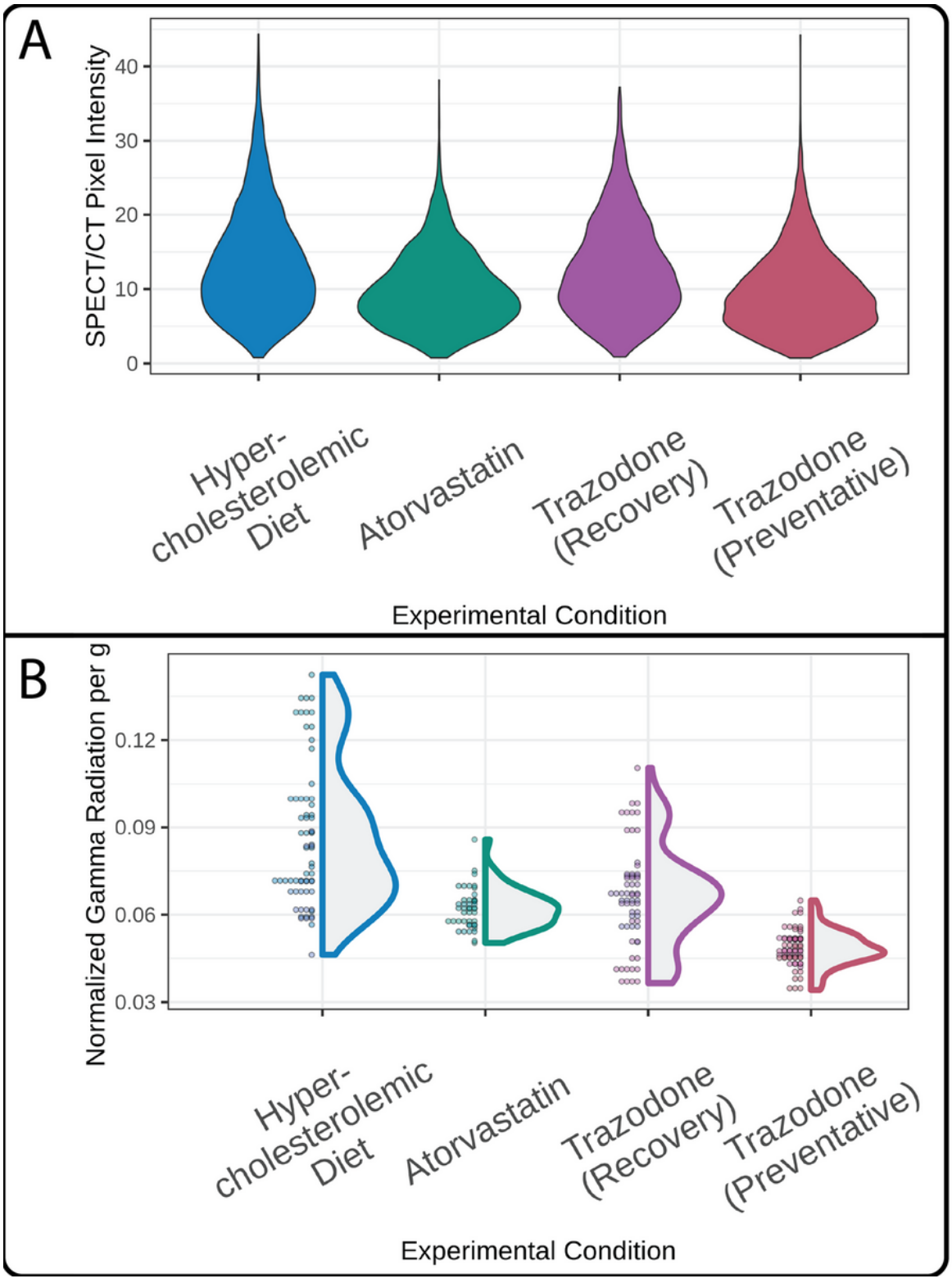

Figure 3

Molecular Imaging of Rabbit Atherosclerotic Lesions. A) Distribution of SPECT/CT pixel intensities by condition, expressed as violin plots. B) Distribution of % injected dose per gram by condition. Each bubble represents the normalized reading from one aorta segment.

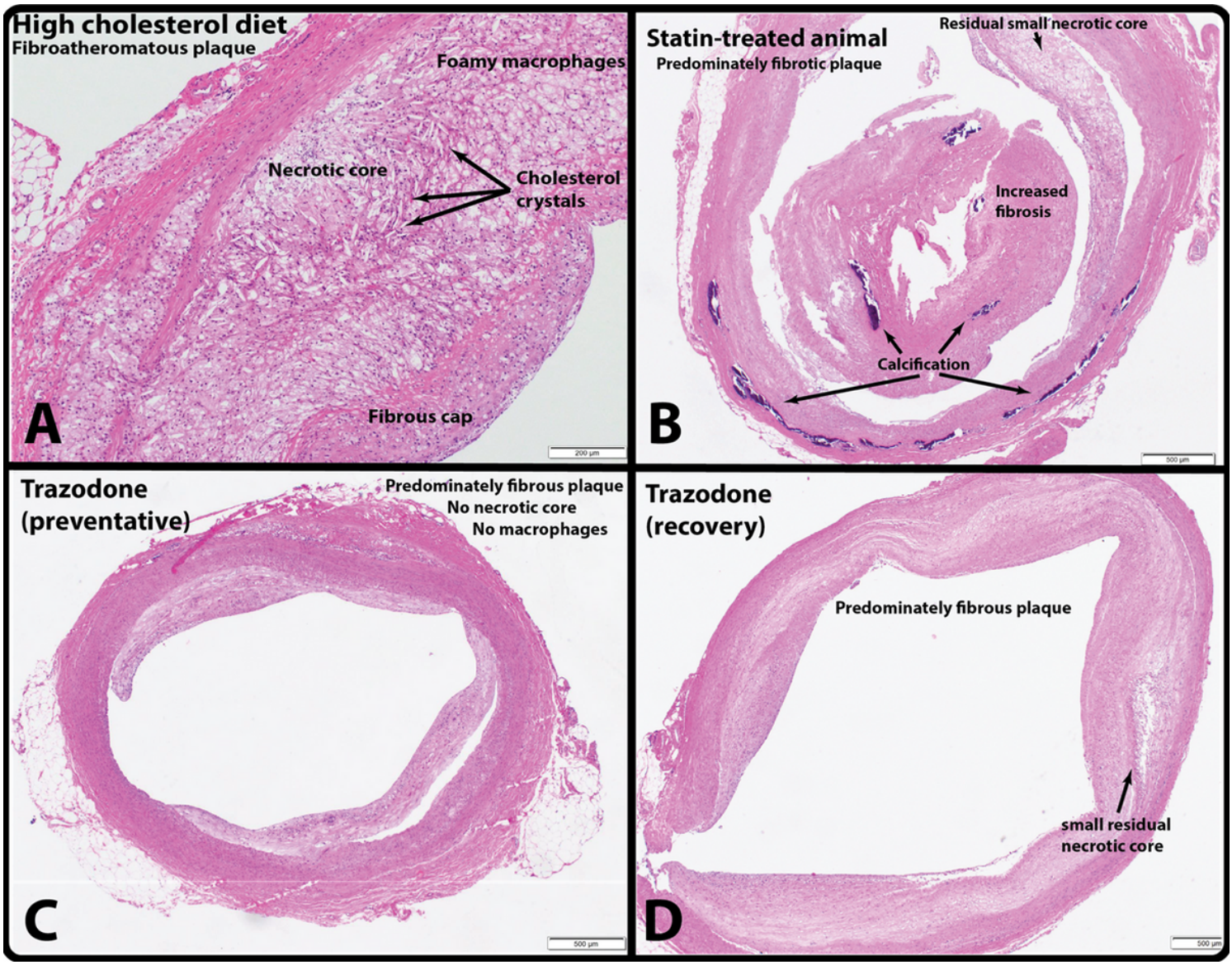

Figure 4

Representative images from histopathological examination of aorta tissue in four experimental conditions. A) Rabbits fed a hypercholesterolemic diet without pharmacological intervention exhibited extensive evidence of atherosclerosis. Pictured is a fibroatheromatous plaque with a large necrotic core, cholesterol crystals, and macrophage invasion. B) Statin-treated animals had less atherosclerosis and elevated levels of fibrosis and calcific lesions. C) Preventative-dose trazodone rabbits had predominately fibrous plaques with little necrotic core or macrophage invasion D) Recovery-dose trazodone rabbits also had predominantly fibrous plaques, although some atherosclerotic lesions also had residual necrotic cores.

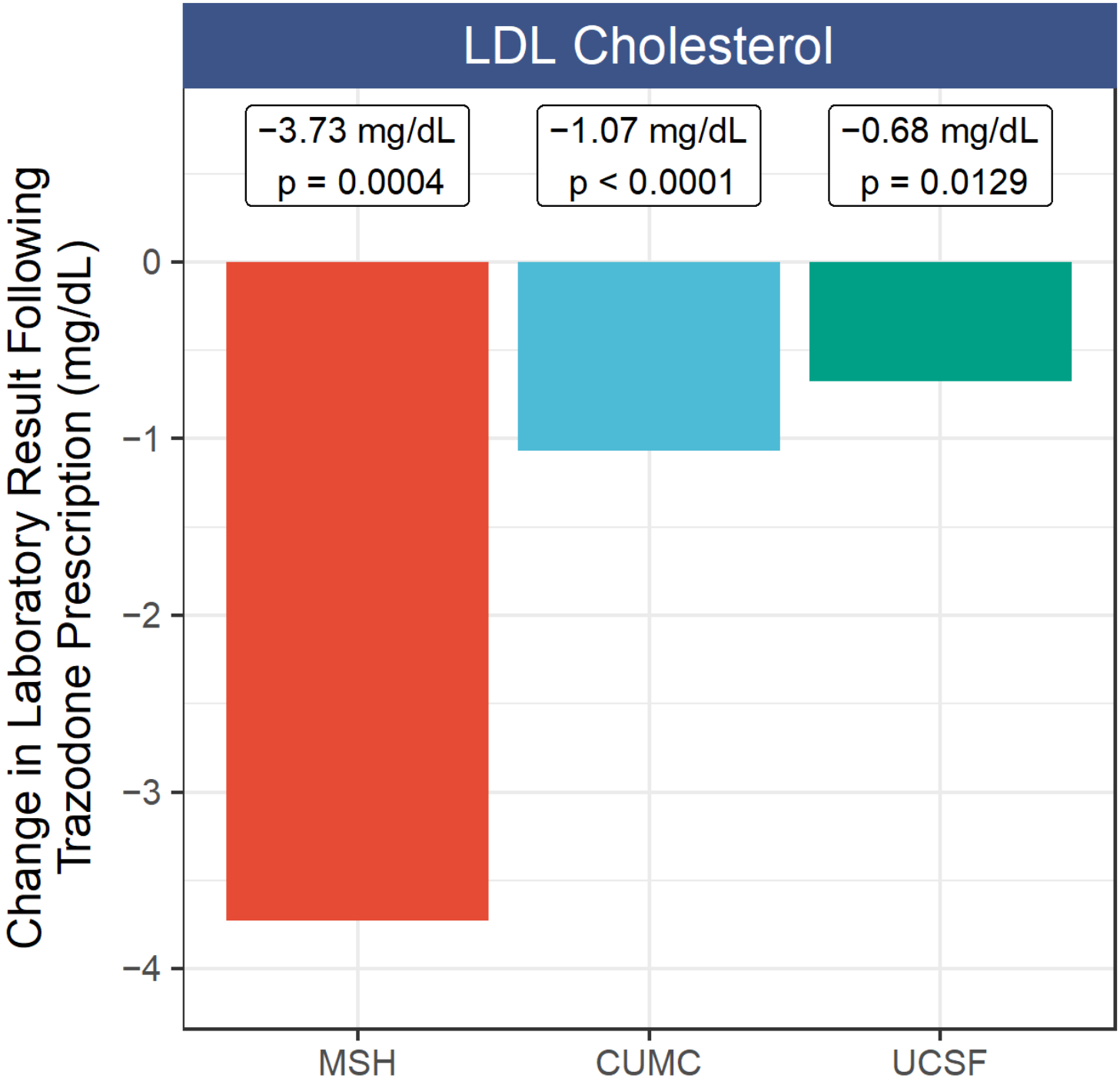

Figure 5

LDL cholesterol laboratory results in the year immediately following trazodone prescription, compared to lipid levels in the preceding year. P values were computed by linear mixed model to account for repeated measurements and intra-individual correlations after adjusting for age, sex, and race/ethnicity.

Central illustration.

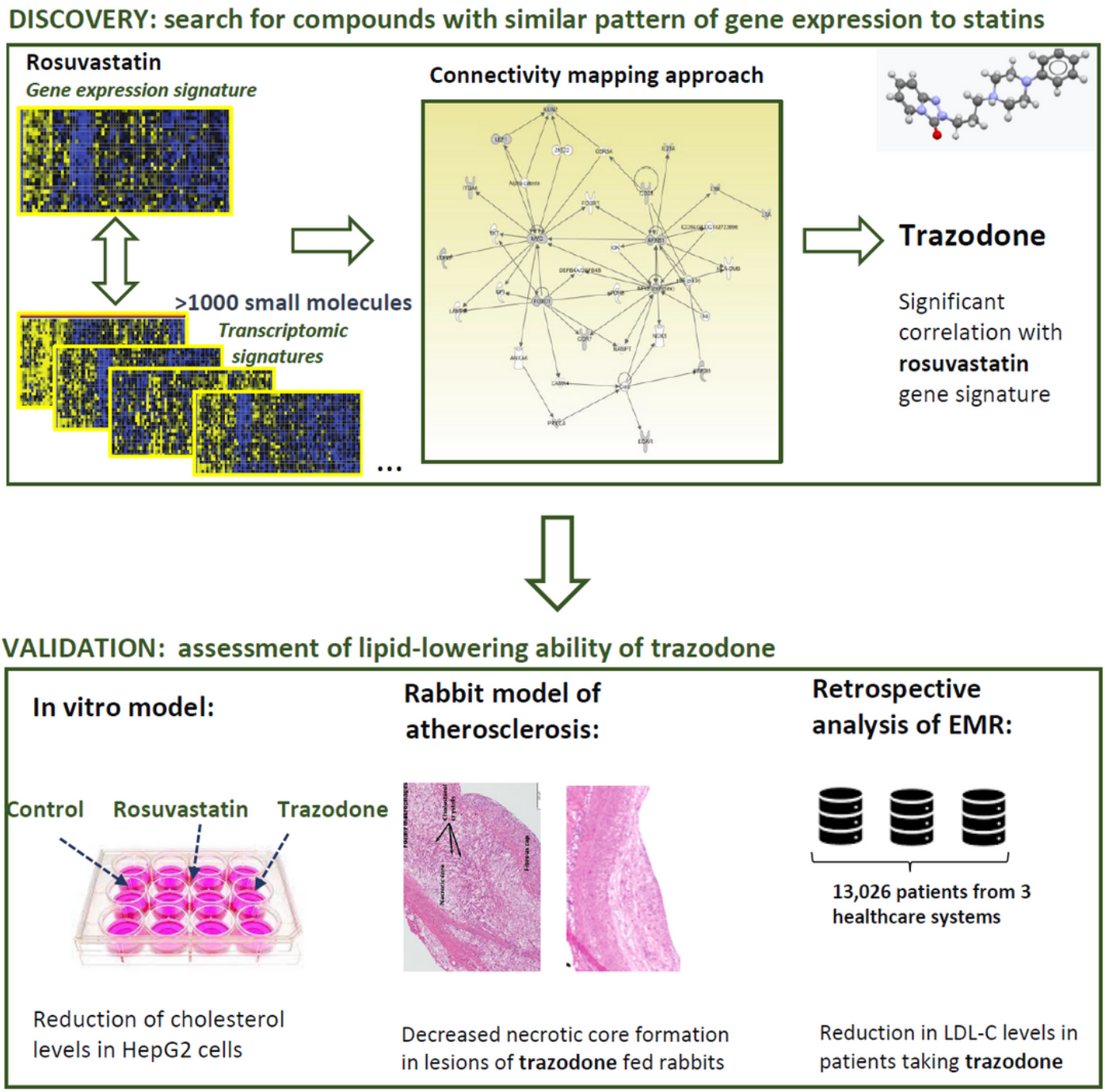

Figure 6

Repurposing the atypical antidepressant trazodone for atherosclerotic cardiovascular disease. We compared transcriptomic signature of high-intensity rosuvastatin therapy derived from a clinical trial (13) to more than 1,039 in-vitro gene expression signatures of cells exposed to drugs using computational bioinformatics. Antidepressant trazodone induced changes in gene expression similar to those observed in human patients undergoing 8–12 weeks of rosuvastatin therapy. Based on the discovery, lipid-lowering

efficacy of trazodone was demonstrated in vitro in human hepatocytes and in a rabbit model of atherosclerosis. Furthermore, our analysis of EMR suggested a potential atheroprotective effect in patients receiving prescriptions for trazodone in three large urban healthcare systems.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalAppendix052721.docx