downstream to TLR4: adaptor proteins MyD88 and TRIF. Simvastatin's ability to down-regulate TLR4 results in the inhibition of all downstream signaling pathways and ultimately attenuates the osteogenic response in aortic valve interstitial cells. These data offer mechanistic insight into a possible therapeutic role for simvastatin in the treatment and/or prevention of aortic stenosis.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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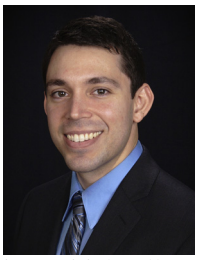
Key Words: aortic, valve, heart, statin, stenosis, calcification, cardiac

Discussion

Dr Joseph Woo *(Stanford, Calif)*. Good morning. I think we'll make our announcements first. On behalf of the WTSA, I welcome you to this morning's concurrent Adult Cardiac Surgery Forum. Evaluations for the annual meeting will be conducted through an online survey. The survey will be sent to all meeting attendees within a week of the conclusion of the annual meeting. Once you complete the brief survey, you will be able to retrieve and print out your CME certificate for your attendance at the annual meeting. The business meeting today at 12:00 pm is for active and senior members only. Immediately following the business meeting is a family luncheon on the Sun and Spa Deck at 12:30. If you have not already signed up for a table at this evening's President's Banquet, you must do so this morning. The seating chart and signup sheets are located in the registration area. All registered attendees must wear their badges to enter this event. This session will end at 8:15 sharp, and the General Session will begin at 8:30. Please keep presentation times to 5 minutes with a 3-minute discussion.

It is my privilege to introduce our first speaker, Michael Jarrett from the University of Colorado. His title is "Simvastatin down-regulates Toll-like receptor 4–induced osteogenic response in human aortic valve interstitial cells."



Dr Michael Jarrett *(Aurora, Colo)*. Dr Woo, members, and guests. Aortic stenosis is the third most common cardiovascular disease. It is exceeded in prevalence only by coronary artery disease and hypertension, yet there is no medical therapy available to treat or prevent this disease. Aortic valve interstitial cells are the predominant resident cell type in valvular leaflets. In response to inflammation, AVICs undergo phenotypic changes and transition from myofibroblasts to inflammatory cells and subsequently to an osteoblast-like cell. The osteogenic phenotype is characterized by increased expression of proteins important for skeletal bone formation such as Runx2, the transcription factor, and ALP, which is an enzyme. Proinflammatory stimulation of Toll-like receptor 4 is an important mechanism by which the osteogenic phenotypic change is induced. TLR4 signals through 2 pathways, MYD88 at the cell surface and TRIF at the endzone. Both pathways activate a common transcription factor, NF-κB that ultimately modulates inflammatory and osteogenic responses in AVICs.

In addition to antilipid properties, statins have anti-inflammatory properties. Previous work by Venardos and colleagues demonstrated that simvastatin inhibits Toll-like receptor 4-induced inflammatory responses in AVICs. We therefore hypothesize that simvastatin may inhibit the TLR4-induced osteogenic phenotypic change as well.

The first thing was to determine if simvastatin inhibits the osteogenic response that is mediated by TLR4. Here we had human primary AVICs and treated with LPS as TLR4 stimulus for a period of 48 hours and with or without simvastatin at 30, 40, and 50 μM concentrations, and then we assessed for expression of Runx2 and ALP. You can see here that both proteins were up-regulated after TLR4 stimulus, and that simvastatin attenuated that response.

We next sought to determine simvastatin's influence on in vitro calcium deposition. Here AVICs were treated with a condition media for a period of 10 days with LPS with or without simvastatin at a 50 μM dose. After that time, calcium deposition staining was performed, and results are shown here. TLR4 stimulus up-regulates in vitro calcium deposition, and simvastatin inhibited that response.

The second aim was to see if simvastatin inhibits NF-κB activation that is TLR4-dependent. Peak NF-κB phosphorylation was found to occur 2 hours after TLR4 stimulus was applied. Therefore, AVICs were treated with LPS with and without simvastatin and phosphorylation of NF-κB assessed. You can see here, simvastatin up-regulated or…excuse me, LPS up-regulated phosphorylation of NF-κB, and simvastatin abrogated that response.

We next sought to determine the role of the adapter proteins, MYD88 and TRIF in terms of mediating the TLR4-dependent osteogenic response. Here we used a lentiviral-mediated knockdown of both MYD88 and TRIF and then treated AVICs with LPS for a period of 48 hours and assessed for expression of Runx2 and ALP. Both proteins here were up-regulated by TLR4 stimulus, and knockdown of both MYD88 and TRIF attenuated that response.

A third and final aim was to see whether simvastatin modulates TLR4 levels in AVICs. Here AVICs were treated with simvastatin, and then TLR4 levels were measured at various time points afterward. Starting at 1 hour, TLR4 was down-regulated, and that response was maintained across all time points. We then followed up with a high- sensitivity ELISA of cell media to see if receptor shedding was occurring, and we were unable to detect TLR4 in cell culture media.

So in summary, simvastatin inhibits the TLR4-induced osteogenic responses in AVICs and attenuates the TLR4-mediated NF-κB activation. Both MYD88 and TRIF-dependent signaling modulate the osteogenic response, and simvastatin negatively regulates TLR4. In light of negative ELISA results, we can't attribute this to receptor shedding, so it is more likely due to increased protein degradation rather than decreased gene expression, which takes longer to affect protein levels.

So in conclusion, simvastatin may have a therapeutic potential in aortic stenosis. Thanks. I'm happy to answer any questions.

Unidentified speaker. So there have been clinical trials looking at statins in order to modify or reduce the calcium burden in aortic valve disease without positive results. Do you think it's just the time point in the progression of the disease, and that if you don't treat early enough, you're just not going to have an impact of pharmacologic therapy?

Dr Jarrett. Thank you for that question. It's a great point, and prior clinical studies have shown largely negative results as you have said. I do, you know, believe that that is the case, so all these patients in these clinical studies had significant valvular disease at the time that they were enrolled, and simvastatin was unable to reverse the disease that was already established, but I think treating a patient at risk for AS or with just mild clinically silent disease with a statin may have, you know, preventative potential, and I think that is where clinical trials should be focused in the future, absolutely.

Dr Tom Burdon *(Stanford, Calif).* Great talk, and congratulations to the Colorado group and David and the basic science pursuit of the inflammation of the aortic valve as a cause.

Dr Jarrett. Thank you.

Dr Burdon. So as statins are sort of swarming our culture and more and more literature comes out every day that everybody should be taking a statin, and a lot of people had been taking statins for a long time, is there any plan in Colorado to sort of somehow dovetail that in to what Richard says, into a clinical project because the time is right now? People have been taking statins for at least 15 years now, and an ever-increasing percentage of our population is taking them.

Dr Jarrett. That's a great question. At this point, we have not made plans to do that. I think it would be a great clinical study to follow up with, you know, in terms of our basic science findings. We have done some animal models that have shown that basically inhibition of inflammation has a positive result, but we haven't done that with statins yet, so I think that would be a great thing to do, we should get organized and try to do that.

Dr Woo. Last question.

Dr Burdon. One quick final question. Do you think that the mechanism of calcification that you've shown is the same in bioprosthetic valves?

Dr Jarrett. The same in what was that?

Dr Burdon. Bioprosthetic valves.

Dr Jarrett. Oh, that's a great question.

Dr Burdon. Should we take everyone and put them on a statin that we put a bioprosthesis in, which has also been tried clinically, but maybe the doses aren't right, or other issues.

Dr Jarrett. I see. So that's interesting, because the pathobiology of AS in a valve that's in vivo, you know, the normal valve is driven by living cells and in the prosthetic valve, those cells are absent or not alive. So I think that we do know that the pathobiology of aortic stenosis involves inflammation, it involves monocytes, it involves other inflammatory cells, and I think that some of these same transcription factors and enzymes are involved, and I think some of the inflammatory cells are involved, but I do think it has to be a different mechanism ultimately. I don't think it's the same, just due to the fact that the interstitial cells are not mediating the calcification in the prosthetic valve. It's the other cells, the inflammatory cells that are doing that.

Dr Woo. Great, well done.

Dr Jarrett. Thank you very much.