Heat Shock Proteins and Endotoxin Combined as a Trigger for Inflammatory Cytokine Release During Cardiopulmonary Bypass: A Possible Third Way?

To the Editor:

As testified to by the report of Dybdahl and colleagues, and the subsequent editorial by Pockley, heat shock proteins (HSPs) are increasingly seen as important mediators in a number of cardiovascular disease states. The former work describes the release of HSP70 and interleukin-6 (IL-6) into the circulation during cardiopulmonary bypass (CPB) as well as the liberation of IL-6 and tumor necrosis factor-α from murine and human mononuclear cells by HSP70 signaling through CD14 and toll-like receptor-4. It is tempting to conclude that the first observation is as a direct consequence of the latter, as the authors speculate, but there are alternative possibilities.

Endotoxaemia is a salient feature of CPB (thought consequent on the translocation of endotoxin across gut mucosa) and has long been associated with pro-inflammatory cytokine liberation in this context. Therefore endotoxaemia alone could explain the increase in IL-6 found in patients undergoing CPB. Furthermore, although Dybdahl et al were careful to use recombinant human HSP70 with low endotoxin content (0.8 to 1.2 EU/mL) in their stimulation work, HSP-bound endotoxin may escape detection by assays of endotoxin activity as well as evade neutralization by polymyxin B (used as a control). The discrepancy between in vivo levels of HSP70 and the concentrations used experimentally (5000 fold difference in this case) may also be a concern to some. This contrasts, for example, with plasma endotoxin activity of around 4 EU/mL (equivalent to 40 ng/mL) during CPB and its relationship to HSP70 levels and onset of the inflammatory response. Nevertheless, repeated in vitro experiments strongly suggest that HSP70 induces proinflammatory cytokines, and similar results have been obtained in other laboratories. In vitro, it may be difficult to rule out that the induction of proinflammatory cytokines is due to lipopolysaccharide bound to HSPs, as discussed by Wallin et al. However, experiments with Polymyxin B and heat inactivation support the suggestion of an inflammatory response dependent on HSPs.

The discrepancy between the in vivo systemic levels of HSP70 and the concentrations needed in vitro is of some concern, and new experiments are necessary to clarify this concern. The experiments published in Circulation were done under serum-free conditions. Preliminary new results from our laboratory show that serum increases the proinflammatory response in monocytes stimulated with HSP70. This may explain some of the discrepancy. In addition, serum may contain components that bind HSP70, thereby reducing the detection of it with immunoassays. This does not necessarily lead to a reduction in biologic activity, and we hope to explore this in the near future. Finally, in vivo, HSP70 could possibly appear in higher concentrations locally, initiating an inflammatory response on site.

We also find the results from Triantafilou et al interesting, as mentioned in our paper. It may be that stress induced by bacterial invasion activates and releases HSPs, which, in turn, increases the inflammatory response. Further research to elucidate receptor clustering and signaling initiated by bacteria as well as HSPs is necessary.

A new study is underway to explore the extent and timing of endotoxaemia during CPB in relation to HSP70 release and onset of the inflammatory response.

Aidan P. Bolger, MRCP, BSc
Sabine Genth-Zotz, MD
Stefan D. Anker, MD, PhD


Response

We appreciate the comments on our article by Dr Bolger, and agree that it cannot be overlooked that endotoxaemia contributes to the inflammatory response in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass (CPB). However, our study clearly shows that heat-shock protein (HSP) 70 is released into the circulation of these patients, and that they have an inflammatory response. Furthermore, the results from Triantafilou et al are interesting, as mentioned in our paper. It may be that stress induced by bacterial invasion activates and releases HSPs, which, in turn, increases the inflammatory response. Further research to elucidate receptor clustering and signaling initiated by bacteria as well as HSPs is necessary.

A new study is underway to explore the extent and timing of endotoxaemia during CPB in relation to HSP70 release and onset of the inflammatory response.

Brit Dybdahl, MD
Egil Lien, PhD
Trude H. Flo, PhD
Terje Espevik, PhD
Anders Sundan, PhD

Faculty of Medicine
Institute of Cancer Research and Molecular Biology
Norwegian University of Science and Technology
Trondheim, Norway

Alexander Wahba, MD
Olav F.M. Sellevold, MD, PhD

Department of Cardiothoracic Surgery
St Elisabeth Heart Center
University Hospital of Trondheim
Trondheim, Norway
Correspondence

Anders Waage, MD, PhD
Department of Medicine
Section for Hematology
University Hospital of Trondheim
Trondheim, Norway

Nilofer Qureshi, PhD
School of Medicine
Director Shock and Trauma Research Center
University of Missouri, Kansas City


