Cell Stress and Chaperones (2015) 20:721–723
DOI 10.1007/s12192-015-0617-9

LETTER TO THE EDITOR

Hsp27 and Hsp70 in chronic obstructive pulmonary disease: certainties vs doubts

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Received: 29 May 2015 / Revised: 3 June 2015 / Accepted: 23 June 2015 / Published online: 21 July 2015

Dear Editor,

We read with great interest the work by Cui et al. (2015) in which they measured the levels of Hsp70 and Hsp27 in plasma and lymphocytes obtained from coal workers (CW) affected by chronic obstructive pulmonary disease (COPD) alone or associated with pneumoconiosis (CWP).

They found that Hsp70 levels were higher in plasma of COPD subjects affected by CWP compared to COPD subjects without CWP and to controls. There was no difference in Hsp70 levels between COPD without CWP and controls. Hsp70 levels in lymphocytes did not show differences among the three groups.

The authors found lower levels of Hsp27 in plasma from patients when comparing controls to both COPD with and without CWP, as well as when comparing COPD groups between them. The lowest levels of plasma Hsp27 were found in the COPD-with-CWP group. By contrast, Hsp27 levels in lymphocytes were higher in COPD with or without CWP compared to controls. No differences were detected between the two COPD groups, with or without CWP.

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These data are partially in agreement with our previous results pertinent to a population of patients affected by COPD without CWP (Cappello et al. 2011). We quantified by immunohistochemistry the levels of Hsp10, Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 in the bronchial mucosa, and we did not find any difference in the histological levels of Hsp27, Hsp70, and Hsp90, whereas there were differences for Hsp10, Hsp40, and Hsp60, when comparing COPD subjects with a control group of non-smokers. Hence, the data for Hsp70 levels in the plasma found by Cui et al. are in agreement with what we have found in the bronchial mucosa. However, the data on Hsp27 are not in agreement with our study or with that of Hacker et al. (2009), who found higher levels of both Hsp27 and Hsp70 in the serum of COPD patients compared to controls.

In our work with bronchial biopsies, we subdivided patients into two subgroups: patients with mild/moderate and patients with severe/very severe COPD. Biopsies were taken after a wash-out period from corticosteroid therapy of 30 days, and we evaluated Hsp levels in the epithelium and lamina propria layers of bronchial mucosa, separately. Our study was similar to the study by Hacker et al. since they also divided patients into two subgroups (mild/moderate and severe/very severe COPD) and their patients underwent a wash-out period of 15 days. By contrast, Cui et al. did not divide patients into subgroups neither their patients underwent a wash-out period from corticosteroids. This could be, in part, responsible for the different results obtained by us and by Cui et al. Furthermore, the discrepancies about Hsp27 and Hsp70 levels in the bronchial mucosa (Cappello et al. 2011), and serum (Hacker et al. 2009), could be explained if Hsp27 and Hsp70 do not originate only in the bronchial mucosa of greater bronchi but also in other cells, e.g., those in peripheral airways or lung interstitium. This would mean that Hsp-level changes in sera from COPD patients could be a similar phenomenon as

that observed in other chronic inflammatory diseases, involving the bowel (Rodolico et al. 2010) or the thyroid (Marino Gammazza et al. 2014) and would render very difficult a direct comparison of results obtained from examining tissue and serum or plasma. We suggest that the discrepancy in the levels of Hsp27 in tissue (as shown by Cappello et al. 2011) and in plasma (as shown by Cui et al. 2015) could be due to various factors, such as (1) they did not sub-divide COPD patients without CWP in two subgroups, as we did; (2) patients in their study did not undergo a wash-out of therapy (corticosteroids? other?); and (3) the levels of Hsps, at least of some of them, in plasma or serum and in bronchial mucosa may not necessarily change in unison and in the same direction in all pathological conditions that show Hsp variations.

To deal with the first point, we reevaluated our data pooling the results from all COPD patients and comparing them to healthy, age-matched non-smoker controls. The new data confirmed that Hsp70 levels were about the same in tissue (bronchial mucosa) in all COPD patients and controls, while Hsp27 levels were significantly higher in COPD patients ($p<0.05$) compared to non-smoker controls. These results are again in contrast with the data obtained by Cui et al., who found lower Hsp27 levels in plasma from patients compared to controls. In our opinion, this discrepancy may be due to the fact that the patients in our study underwent a wash-out before sampling (biopsy) and the patients in the study by Cui et al. did not. Another possible explanation could be that Cui et al. selected their controls from a population of age-matched healthy coal workers who, although "healthy," must have been exposed to coal dust. This selection criterion could complicate the interpretation of the results. Unfortunately, the methods to measure Hsp levels by ELISA in serum by Hacker et al. and in plasma by Cui et al. were different and the results (in terms of pg/ml and ng/ml, respectively) cannot be compared to each other. Also, it is reasonable to assume that CW subjects have higher basal levels of Hsps compared to a normal population not exposed to coal dust, and this fact could complicate even more the interpretation of the results.

Interestingly, in the paper by Cui et al., the low Hsp27 levels in plasma were accompanied by higher levels of Hsp27 in lymphocytes from both COPD groups as compared to controls. They hypothesised that Hsp27 could have a regenerative feedback effect in lymphocytes, but we are not entirely convinced by this explanation. Hsp27 has been recently found to have a role in lymphocytes in the maintenance of the functional activities of glutathione reductase and glutathione peroxidase, and inhibition of Hsp27 in lymphocytes induced programmed death in these cells (Ryazantseva et al. 2015). In our opinion, to better characterize the lymphocyte population in the COPD patients, it would be useful to determine if Hsp27 is low in all lymphocyte subgroups (CD4, CD8, etc.) or just in selected subpopulations. Again, a wash-out from anti-inflammatory treatments preceding the lymphocyte testing could significantly influences the results.

In summary, the paper by Cui et al. opens interesting questions about Hsp27 and Hsp70 in COPD, such as:

1. Can the levels of these proteins be used as COPD biomarkers? We need further data on a larger population of COPD patients and healthy controls, appropriately selected and enrolled, to obtain a satisfactory answer to this question.
2. Can variations in Hsp27 and Hsp70 plasma levels let us to postulate a role of these proteins in COPD pathogenesis? If so, it needs to be confirmed by further in vitro and in vivo (i.e., animal models) studies, measuring the effects of the stimulation with Hsps on airway cells in terms of cytokine production and histological changes in the bronchial mucosa.
3. Can Hsps be used in COPD therapy? As discussed recently at the VIIth International Symposium on Heat Shock Proteins in Biology & Medicine (Calderwood and Hightower 2015), Hsps may have both pro- and anti-inflammatory effects, depending on their levels (doses), the microenvironment, and whether they are released in free-soluble form or bound to the membrane of exosomes. Since Hsps are in general protective, one can conclude that overall they work in the fine-tuning of inflammation, firstly by stimulating inflammation when there is a lesion in a tissue that needs to be regenerated, and secondly, by moderating the inflammatory process if necessary to prevent the damage that might be caused by excessive inflammation. However, sometimes something may go wrong and Hsps trigger or perpetuate a chronic inflammation.

Our most immediate goal remains to understand whether and how we can use Hsps (or their inhibitors) in the treatment of chronic inflammatory diseases, such as COPD. At present, we are still far from that target but the studies discussed here provide useful clues on how to proceed.

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