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Title: Carbocysteine restores the expression and the activation of TLR4 altered by cigarette smoke extracts in bronchial epithelial cells

Dr. Elisabetta 5309 Pace pace@ibim.cnr.it MD [1], Dr. Maria 5310 Ferraro ferraro@ibim.cnr.it [1], Dr. Liboria 5311 Siena siena@ibim.cnr.it [1], Dr. Stefania 5312 Gerbino stefania1201@libero.it [1], Dr. Caterina 5313 Di Sano disano@ibim.cnr.it [1], Dr. Serena 5314 Di Vincenzo serenadivincenzo@libero.it [1], Dr. Rossella 5315 Balsamo Rossella.Balsamo@dompe.it MD [2], Dr. Luigi 5316 Lanata Luigi.Lanata@dompe.it MD [2] and Dr. Mark 5318 Gjomarkaj gjomarkaj@ibim.cnr.it MD [1]. [1] IBIM, Consiglio Nazionale Delle Ricerche, Palermo, Italy and [2] Medical Affair, Dompé SPA, Milan, Italy .

Body: Cigarette smoke represents the major risk factor for chronic obstructive pulmonary disease (COPD). Cigarette smoke extracts (CSE) alter TLR4 expression and activation in bronchial epithelial cells. Carbocysteine, an anti-oxidant and mucolytic agent, is effective in reducing the severity and the rate of exacerbations in COPD patients. The effects of carbocysteine on TLR4 expression and on the TLR4 activation downstream events are largely unknown. This study was aimed to explore whether carbocysteine, in a human bronchial epithelial cell line (16-HBE), counteracted some pro-inflammatory CSE-mediated effects. In particular, TLR4 expression, LPS binding in CSE -stimulated 16-HBE and actin reorganization in neutrophils cultured with supernatants from bronchial epithelial cells which were stimulated with CSE and/or carbocysteine were assessed. TLR4 expression and LPS binding were assessed by flow cytometry. Actin reorganization a prerequisite for cell migration, was determined using Alexa Fluor 488 phalloidin in neutrophils by flow cytometry and confocal microscopy. CSE increased TLR4 expression and LPS binding and increased neutrophil chemotactic activity. Carbocysteine in CSE stimulated bronchial epithelial cells, reduced TLR4 expression, LPS binding and neutrophil chemotactic activity. In conclusion, the present study provides compelling evidences that carbocysteine may contribute to control the inflammatory processes present in smokers.