Ćurčić *et al.: Intrinsic Activity*, 2015; **3**(Suppl. 2):A3.1
http://www.intrinsicactivity.org/2015/3/S2/A3.1
published online: 9 September 2015

21st Scientific Symposium of the Austrian Pharmacological Society: Joint Meeting with the British Pharmacological Society and the Pharmacological Societies of Croatia, Serbia and Slovenia

Graz, 16–18 September 2015

MEETING ABSTRACT

A3.1

Neutrophil effector responses are fully suppressed by secretory phospholipase A_2-modified HDL

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Background: Secretory phospholipase A_2 ($sPLA_2$) generates bioactive lyso-phospholipid products implicated in atherosclerosis. In patients with acute coronary syndrome, the $sPLA_2$ inhibitor varespladib surprisingly increased the risk of myocardial infarction. High-density lipoprotein (HDL) is the main source of phospholipids and the major substrate for $sPLA_2$ in plasma. Therefore, we investigated the effects of $sPLA_2$-mediated modification of HDL on neutrophil function, a critical player in atherosclerosis and inflammation.

Methods: Human neutrophils were isolated from peripheral blood of healthy human volunteers. The neutrophil shape change, CD11b activation and Ca^{2+} flux were measured by flow cytometry. Neutrophil adhesion was measured under flow conditions using the flow-chamber assay. Lipid rafts were stained with FITC–cholera toxin B and its abundance was assessed by flow cytometry and fluorescent microscopy. Cholesterol efflux was measured from neutrophils pre-loaded with [^{3}H]cholesterol.

Results: Treatment of HDL with $sPLA_2$ ($sPLA_2$-HDL) resulted in the formation of palmitoyl-lysophosphatidylcholine (LPC 16:0) as the most prominent LPC species. $sPLA_2$-HDL rapidly prevented neutrophil shape change, Ca^{2+} flux, CD11b activation, adhesion, migration and formation of neutrophil extracellular traps (NETs). Moreover, $sPLA_2$ treatment of HDL markedly increased cholesterol efflux capability of HDL associated with a rapid disruption in cellular cholesterol-rich microdomains (lipid rafts). Native HDL showed no significant effects and removing LPC products from $sPLA_2$-HDL abolished all anti-inflammatory activities towards neutrophils, whereas enrichment of native HDL with LPC 16:0 mimicked $sPLA_2$-HDL effects.

Discussion: Overall, our studies suggest that the increased cholesterol-mobilizing activity of sPLA-HDL and suppression of rise in intracellular Ca^{2+} levels are the likely mechanism that counteracts agonist-induced activation of neutrophils. Our results raise the possibility that $sPLA_2$-induced modification of HDL composition and function modulates neutrophil trafficking and effector responses during inflammation.

Acknowledgements: The work was supported by the Austrian Science Fund FWF (P22976 and P22521) and the Austrian National Bank (no. 14853)

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