C57Bl6 mice (male, aged 8–12 weeks, 8–12/group). After 21 days sponges were excised and fixed, and vessel growth and collagen deposition were assessed by counting and picro-sirius red staining, respectively. At equipotent anti-inflammatory doses, corticosterone decreased the amount of collagen in sponges (3 mg changing to 30%±8% of control; $p<0.05$) whereas 5αTHB did not (15 mg; 128%±20%). Similarly, while corticosterone reduced new vessel growth to 15%±3% of control ($p<0.05$), this effect was less marked with 5αTHB (46%±7%; $p<0.05$) vs corticosterone.

In the second model, vessel growth was stimulated from mouse aortic rings (C57Bl6 mice, male, age 8–10 weeks) and attenuation of growth was assessed following incubation with steroid (dose ranges 1 nM–10 μM, n=6–8/dose). Angiogenesis was suppressed to a lesser extent by 5αTHB (EC50 2399 nM) than by dexamethasone (8 nM) or hydrocortisone (675 nM; n=6–8/dose).

These data suggest that 5αTHB could provide a safer anti-inflammatory treatment for topical application. It has fewer adverse effects on angiogenesis and collagen deposition than hydrocortisone and is thus likely to be less detrimental during wound repair.

23 NADPH OXIDASE 5 (NOX5), CHOLESTEROL-RICH MICRODOMAINS AND ANG II SIGNALLINGSIGNALING IN HUMAN PRIMARY VASCULAR SMOOTH MUSCLE CELLS

A Anagnostopoulou*, P Persson, A Montezano, R Touyz. *BHF Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, 126 University Place, Glasgow, UK*

10.1136/heartjnl-2017-311433.23

Background and hypothesis Nox5 has been identified in human vessels. However the trafficking, regulation and function of vascular Nox5 is unclear. We questioned the function of Nox5 and other Nox isoforms in signalling associated with vascular smooth muscle cell contraction, growth and cytoskeletal organisation and assessed whether lipid rafts/caveolae play a role in Nox5 regulation.

Methods Human primary vascular smooth muscle cells (VSMCs) isolated from small arteries were studied. Nox isoforms were sequentially downregulated by siRNA. siRNA to p22phox reduced activity of Nox1,2 and 4, since these isoforms are p22phox-dependent. Nox5 was targeted by Nox5 siRNA. Signalling pathways associated with contraction (MLC, MYPT1), cell growth (PCNA, p53) and cytoskeletal organisation (Ezrin-Radixin-Moiesin) were assessed by immunostudies. Cells were stimulated with Ang II in the absence and presence of disrupters and enhancers of cholesterol-rich microdomains. Nox5 interaction with lipid rafts/caveolae was examined by immunohistochemistry in intact vessels and by sucrose gradient cell fractionation in cultured cells.

Results Nox5 inhibition by siRNA was associated with reduced Ang II-induced phosphorylation of MLC20 (by 0.74-fold, 5 mins stimulation) and MYPT1 (by 0.5-fold, 5 mins stimulation). p22phox siRNA did not significantly alter activation of MLC20 or MYPT1. siRNA to p22phox and Nox5 decreased Ang II-induced increase in PCNA expression (by 0.18-fold and 0.3-fold in Ang II 2 hour and 8 hour stimulation respectively, $p<0.05$) and decreased phospho-p53 levels (by 0.4-fold in Ang II 5 mins stimulation, $p<0.05$). In isolated human small arteries Nox5 co-localised with caveolin-1 as assessed by immunohistochemistry. In fractionated cells, Nox5 and Nox1 localised in fractions 3 and 4, which are rich in cholesterol microdomains. To further explore the role of lipid-rafts/caveoale in Nox function, we disrupted lipid-rafts/caveolae using methyl-b-cyclodrexin (MCD) and nystatin. MCD and nystatin increased Ang II-induced Nox-derived superoxide formation and increased Ang II-induced phosphorylation of MLC20 (by 3.45-fold and 1.12-fold respectively) and Ezrin-Radixin-Moiesin (by 1.2-fold in MCD, Ang II 15 mins stimulation and 1.2-fold in nystatin, Ang II 5 mins stimulation).

Conclusions These results indicate that in human VSMCs Ang II stimulates contractile pathways through Nox5-dependent pathways whereas signalling associated with VSMC growth and cytoskeletal organisation involve multiple Nox isoforms. Nox5 co-localises in lipid rafts/caveolae, which may play a role in Nox5 trafficking. Our data identify novel redox signalling processes through cholesterol-rich microdomains and highlight a potentially important function of Nox5 in vascular contraction.