

## **Inhibition of nitric oxide synthase (NOS) reduces the effect of stress hormone signalling in breast cancer**

Renee L. Flaherty\*, Bhavik A. Patel\*, Marcus C. Allen\*, Melanie S. Flint\*

\*School of Pharmacy and Biomolecular Sciences, University of Brighton, Moulsecoomb, Brighton, BN2 4GJ, UK

Keywords: breast cancer, nitric oxide, DNA damage

Expression of nitric oxide synthase (NOS) has been found to correlate with tumour progression in breast cancer, indicating that NO activity may drive malignant growth. Previously we have shown that the stress hormone cortisol acts through a nitric oxide synthase (NOS) mediated pathway to induce production of nitric oxide (NO), and can induce DNA damage in breast cancer.

Breast cancer cell lines MCF-7 and MDA-MB-231 as well as the mouse mammary tumour cell line 66CL4 were exposed to cortisol and levels of intracellular NO were measured using composite electrochemical sensors. DNA damage was quantified using immunofluorescence and expression of iNOS and metastatic markers VEGF and TWIST were examined using qPCR. An *in vivo* syngeneic breast cancer model was also used to examine the effect of L-NAME, a NOS inhibitor, on tumour aggressiveness and metastasis in conjunction with daily restraint stress (2hrs) (n=4/group repeated in duplicate).

Cortisol significantly increased the expression of iNOS, the generation of NO and DNA damage in breast cancer cells and this was blocked by the NOS inhibitor L-NAME. A significant increase in VEGF and TWIST expression was also observed in response to cortisol. Furthermore, L-NAME also significantly reduced primary tumour growth in stressed mice and reduced the number of metastatic sites/mouse. Tumour microvasculature (as evidenced by CD31 expression) was significantly increased in stressed mice and this was reduced with L-NAME treatment.

We demonstrated that L-NAME through inhibition of NO signalling is effective in reducing primary tumour formation and metastatic potential in stressed mice. This data may have impact for patients with breast cancer experiencing extreme stress and further genomic analysis are ongoing.