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Asymmetric dimethylarginine negatively influences osteoblast differentiation *in vitro*

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Asymmetric and symmetric dimethlarginine (ADMA and SDMA) are endogenously occurring methylarginines released during protein turnover. ADMA inhibits all isoforms of nitric oxide and elevated plasma concentrations alter gene expression in cardiovascular disorders as determined both *in vitro* and *in vivo* (Smith *et al* 2005, Leiper *et al* 2007), SDMA has no effect on these parameters. ADMA has also been shown to modulate the bone morphogenetic protein (BMP) signalling pathway (Smith *et al.* 2005), a pathway with a critical role in the differentiation of mesenchymal stem cells. Here we have evaluated whether pathophysiological levels of ADMA could influence *in vitro* osteoblast differentiation.

The pre-osteoblast cell line MC3T3 (1 x 10^4 cells) was cultured in alpha-MEM media (containing 10 % FCS) and differentiated by addition of beta-glycerophosphate (10 mM) and L-ascorbate (50 µg/ml) for 18 days either in the presence or absence of ADMA (5 µM). The culture media was exchanged every three days and cells were also harvested. Cells were then either lysed for alkaline phosphastase activity assays or the total RNA extracted for analysis by northern blotting. Secreted ADMA and SDMA were measured in the control cells throughout differentiation by mass spectrometry (Mookerjee *et al*. 2007). Data is mean ± SD of n=4-6 experiments and *P<0.05 vs appropriate control (data for day 15 is shown below).

Differentiation of MC3T3 cells, as measured by alkaline phosphatase activity, started at day 6 and peaked by day 18. In naïve non-differentiated MC3T3 cells equimolar concentrations of ADMA (0.42 ± 0.015 µM) and SDMA (0.43 ± 0.021 µM) were secreted into the culture media. Differentiated cells at day 15 released 450 % more ADMA (1.89 ± 0.085 µM, n=4) than SDMA (0.51 ± 0.042 µM, n=4), P=0.028.

We next evaluated the effect of culturing cells in pathophysiological concentrations of ADMA, by day 15 the cells grown in the presence of ADMA (5 µM) had 21.32 ± 7.75 % (p=0.049, n=6) less alkaline phosphatase activity and displayed a 2.19 ± 0.35 fold higher expression of BMP inducible kinase (p = 0.022, n=6) corrected for β-actin. Nitrite secretion by MC3T3 cells was reduced by day 15 in the presence of ADMA (5.15 ± 0.25 µM to 4.266 ± 0.20 µM, P=0.02, n=6), measured by Greiss assay.

In summary, pathophysiological concentrations of ADMA appear to diminish the differentiation of MC3T3 cells and these changes may be partially mediated by the induction of the inducible BMP kinase, which attenuates osteoblast differentiation (Kearns *et al* 2001). Surprisingly the ratio of ADMA: SDMA increases significantly during the osteoblast differentiation, therefore indicating that ADMA may play a role in regulating osteoblast differentiation.

Kearns AE *et al.* (2001) *J. Biol. Chem* 276: 42213-8

Leiper J. *et al.* (2007) *Nature Medicine* 13: 198-203

Mookwejee RP *et al.* (2007) *Hepatology* 45: 62-71

Smith CL *et al.* (2005) *PLoS Medicine* 1031-1043