Effects of Nitric Oxide on Aortic Valve Calcification *in vitro*

Xiang Hua

MBBS

THE THESIS SUBMITTED TO THE ADELAIDE UNIVERSITY FOR THE DEGREE OF MASTER OF CLINICAL SCIENCE

THE WORK DESCRIBED WAS PERFORMED WITHIN THE DEPARTMENT OF CARDIOLOGY, THE QUEEN ELIZABETH HOSPITAL, THE UNIVERSITY OF ADELAIDE

CONTENTS

ABSTRACT

Aims: Aortic stenosis (AS) is characterized by accelerated aortic valve (AV) calcification, but the pathogenesis of this process is poorly understood. The aim of this study was to evaluate the potential impact of NO supplementation on valve matrix calcification in a tissue culture model, and the interaction between superoxide anion release and the process.

Methods: Interstitial cells were isolated from porcine AV leaflets and grown to 95% confluence in 10% serum. Medium was changed to low serum (0.67%) ± transforming growth factor-beta1 (TGF-β1) (5ng/ml) ± study drugs, replenished every 48 h for 4-14 days. Both spontaneous nodule formation and that induced by TGF-β1 over 4-14 days. Experiments were conducted in triplicate in at least 4 cultures at cell passages 4. Nodules were counted by an observer blinded to treatment. In parallel experiment, dihydroethidium staining was utilized to measure superoxide formation in pig aortic valve fibroblasts just to parallel to nodule formation.

Results: Exogenous TGF-β1 elicited a marked increase in calcific nodule formation compared to paired controls. This was inhibited by co-incubation with the NO donor DETA-NONOate (1-100μM), 8-Br-cGMP (1mM) and by the superoxide scavenger, TEMPOL (100μM). In addition, L-NAME (100μM) had no effect on TGF-β1 induced nodule formation. TGF-β1 elicited a marked increase in intracellular superoxide formation compared to paired controls. DETA-NONOate (20μM) and TEMPOL (100μM) blocked intracellular superoxide formation in the presence of TGF-β1.

Conclusions: From these data, we have established that TGF-β1 both induced superoxide anion release and calcific nodule formation in this preparation, while NO donor DETA-NONOate reverses both these effects and cGMP analogue inhibits nodule formation. Furthermore, TEMPOL, a superoxide scavenger, also limits nodule

formation. Therefore, these data suggest that superoxide anion release, induced by TGF-β1 contributes to calcific nodule formation, and that NO can limit this process possibly via the production of cGMP. It remains to be determined whether these effects of NO are relevant to the impact of valvular endothelial dysfunction and/or to a potential therapeutic role for NO donors in prevention of AS.