The Expression, Regulation and Effects of Inducible Nitric Oxide Synthase in Hibernating Myocardium

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# TABLE OF CONTENTS

THESIS SUMMARY................................................................................................................. xii
DECLARATION.......................................................................................................................... xvi
ACKNOWLEDGEMENTS........................................................................................................... xvii

CHAPTER ONE – Introduction

1.1  Coronary Artery Disease and Left Ventricular Dysfunction............................................. 2
   1.1.1  Coronary Artery Disease.............................................................................................. 2
       Epidemiology..................................................................................................................... 3
       Clinical Syndromes........................................................................................................... 4
       Treatment ........................................................................................................................... 6
       Prognosis .......................................................................................................................... 10
   1.1.2  Left Ventricular Dysfunction....................................................................................... 11
       Epidemiology..................................................................................................................... 12
       Clinical Presentation.......................................................................................................... 13
       Functional Classification.................................................................................................... 15
       Treatment of chronic congestive cardiac failure............................................................... 15
       Prognosis.......................................................................................................................... 18
1.2 Hibernating myocardium ................................................................. 20
  1.2.1 Definition ................................................................................. 20
  1.2.2 Prevalence of hibernating myocardium .................................. 23
  1.2.3 Histology ................................................................................ 24
  1.2.4 Biochemical Characteristics .................................................... 27
      Cardiac metabolism at rest .......................................................... 27
      Cardiac metabolism during ischaemia ........................................ 27
      Cardiac metabolism in hibernating myocardium ...................... 28
  1.2.5 Treatment ............................................................................... 30
      Medical or Surgical Treatment .................................................. 30
      Timing of Revascularisation ...................................................... 31

1.3 Detection of Hibernation Myocardium .......................................... 33
  1.3.1 Recovery Post Revascularisation .............................................. 33
  1.3.2 Positron Emission Tomography .............................................. 34
  1.3.3 Dobutamine Echocardiography .............................................. 35
  1.3.4 Single Photon EmissionComputed Tomography .................... 38
      203Thallium ................................................................................. 38
      99mTechnetium (99m Tc) labelled perfusion agents .................. 39
      18FDG SPECT ........................................................................... 42
      123I BMIPP ............................................................................. 42
      Hypoxia markers ...................................................................... 44

1.4 Inducible Nitric Oxide Synthase ..................................................... 45
  1.4.1 Introduction ............................................................................ 45
1.4.2 Regulation of iNOS Expression ........................................... 47
   Induction of iNOS by cytokines ........................................... 47
   The iNOS promoter region ................................................. 49
   The Hypoxia Responsive Element ........................................ 54
   The Antioxidant Responsive Element .................................... 56
1.4.3 Role of iNOS in Myocardial Pathology ................................. 57
   iNOS in Myocardial Infarction ........................................... 58
   iNOS in ischemic preconditioning ....................................... 58
   iNOS in Cardiac Failure .................................................. 59
   iNOS in septic shock ...................................................... 60
1.4.4 Expression of iNOS in Hibernating Myocardium ....................... 60
1.5 Nitric Oxide and Myocardium .............................................. 61
   1.5.1 NO and negative inotropy ........................................... 61
   1.5.2 NO and mitochondrial function .................................... 62
      NO and the respiratory chain ........................................ 62
      Effect of peroxynitrite .............................................. 63
      Relation to myocardial function .................................... 64
      Cell death .............................................................. 64
1.6 Apoptosis ........................................................................ 65
   1.6.1 Definition ............................................................. 65
   1.6.2 Mechanisms of apoptosis .......................................... 67
      Initiation of apoptosis ................................................ 67
      Caspases as the executioners of apoptosis ......................... 68
CHAPTER TWO – Methods

2.1 Isolation of neonatal rat ventricular myocytes .................................................. 84
2.2 Hypoxic experimental conditions ........................................................................ 86
2.3 Lactate assay ....................................................................................................... 88
2.4 Adenosine Triphosphate (ATP) assay .................................................................. 92
2.5 Extraction of cellular protein for caspase 3 activity assay ................................. 93
2.6 Extraction of total RNA ....................................................................................... 94
2.7 Reverse Transcription Polymerase Chain Reaction (RT PCR) .............................. 95
  2.7.1 Reverse transcription ..................................................................................... 95
  2.7.2 Polymerase chain reaction ............................................................................ 96
2.8 Primers and probes ............................................................................................ 97
  2.8.1 Primers ........................................................................................................ 97
      Inducible nitric oxide synthase ........................................................................... 98
      iNOS .................................................................................................................... 98
Hypoxia Inducible Factor 1α ................................................................. 99
Bax ........................................................................................................ 99
Bcl-2 ...................................................................................................... 99
Tumour necrosis factor α ................................................................. 100
Interleukin 1β .................................................................................... 100
Interleukin 6 ..................................................................................... 101
2.8.2 Probes ........................................................................................ 101
Inducible nitric oxide synthase ....................................................... 102
Hypoxia Inducible Factor 1α ............................................................... 102
IRS ...................................................................................................... 103
Bax ........................................................................................................ 103
Bcl - 2 ............................................................................................... 104
Interleukin 6 ..................................................................................... 104
Labeling of RNA probes .................................................................. 105
2.9 Northern hybridisation ............................................................... 108
RNA gel electrophoresis .................................................................. 108
RNA marker ..................................................................................... 109
Transfer ............................................................................................. 109
Hybridization .................................................................................. 110
Chemiluminescence detection .......................................................... 111
2.10 Quantitation of Northern Blots ................................................... 112
2.11 Electromobility Shift Assay ....................................................... 113
2.11.1 Nuclear protein extract ............................................................ 114

vi
CHAPTER THREE – A Cellular Model of Hibernating Myocardium

3.1 Models of hibernating myocardium ......................................................... 124

3.2 Primary cultures of neonatal rat ventricular myocytes .............................. 125

3.2.1 Spontaneous beating in primary cultures of neonatal cardiac myocytes ................................................................. 126

3.2.2 Effect of plating density on neonatal rat ventricular myocytes cultures ........................................................................ 127

3.2.3 Effect of fibroblasts on neonatal rat ventricular myocyte cultures .... 129

3.2.4 Effect of age in culture ........................................................................ 131

3.3 A cellular model of hibernating myocardium ......................................... 134
3.3.1 Effect of chronic hypoxia on spontaneous beating ............................................ 136

3.3.2 Effect of dobutamine on spontaneous beating .................................................... 141

3.3.3 The effect of chronic hypoxia on lactate production ......................................... 144

3.3.4 The effect of chronic hypoxia on ATP production .............................................. 147

3.4 Discussion .............................................................................................................. 149

CHAPTER FOUR – Expression of iNOS in Chronic Hypoxia

4.1 Introduction .............................................................................................................. 155

Studies in whole rats .................................................................................................... 155

Studies in primary cultures of neonatal rat ventricular myocytes ................................... 155

4.2 Effect of hypoxia on iNOS expression in myocyte cultures from Day 1-3
neonatal rats .................................................................................................................. 157

4.2.1 Methods .............................................................................................................. 157

4.2.2 Results ............................................................................................................... 159

4.3 Effect of hypoxia on iNOS expression in myocyte cultures from Day 7
neonatal rats .................................................................................................................. 159

4.4 Effect of hypoxia on iNOS expression in myocyte cultures from Day 5 and Day
4 neonatal rats ............................................................................................................... 165
CHAPTER FIVE - Regulation of iNOS Expression in Chronic Hypoxia

5.1 Introduction ................................................................................................. 174

Transcription factors involved in iNOS regulation under hypoxic conditions
.......................................................................................................................... 176

5.2 Expression of cytokines in chronic hypoxia ................................................. 177

5.3 Transcription factors binding to the iNOS promoter region in chronic
hypoxia.................................................................................................................. 181

5.3.1 Methods .................................................................................................... 181

5.3.2 Hypoxia Responsive Element binding in hypoxia ........................... 182

5.3.3 Binding to the NFκB site under hypoxic conditions ......................... 185

5.4 Expression of Hypoxia Inducible Factor 1 in Chronic Hypoxia ............. 185

5.5 Discussion .................................................................................................. 191

CHAPTER SIX – Effects of iNOS Expression in Chronic Hypoxia

6.1 Introduction ................................................................................................ 195

The role of iNOS in ischemic preconditioning .............................................. 195
iNOS and apoptosis ......................................................................................... 195
6.2 The role of iNOS in the downregulation of contraction in chronic hypoxia... 197
6.3 Apoptosis in Chronic Hypoxia ..................................................................... 198
   6.3.1 Detection of apoptosis in cardiac myocytes ........................................ 198
   6.3.2 Apoptosis in normoxic cells in culture .............................................. 198
   6.3.3 The effect of hypoxia on apoptosis of cardiac myocytes .................... 200
6.4 Role of iNOS in hypoxia-induced apoptosis ............................................... 202
6.5 Confirmation of apoptosis in cardiac myocytes by Annexin V staining ....... 208
6.6 Expression of apoptotic proteins in chronic hypoxia ................................. 212
   6.6.1 Expression of Bax by cardiac myocytes under hypoxic conditions .......................... 212
   6.6.2 Expression of Bcl-2 by cardiac myocytes under hypoxic conditions .............. 215
6.7 Discussion .......................................................................................................... 218

CHAPTER SEVEN – Discussion

7.1 Validity of The Cellular Model of Hibernating Myocardium ..................... 223
7.2 iNOS expression in Chronic Hypoxia .......................................................... 227
7.3 Regulation of iNOS expression under hypoxic conditions ......................... 229
7.4 Effects of iNOS expression under hypoxic conditions ............................... 231
7.5 The possible role of iNOS in hibernating myocardium ............................... 233
Hibernating myocardium involves the downregulation of regional ventricular contraction, leading to cardiac failure, in response to severe coronary stenoses with recovery of function following revascularisation. This results in improved survival. Experiments described in this thesis address the potential role of inducible nitric oxide synthase (iNOS) in hibernating myocardium. Specifically it was sought to establish a cellular model of hibernating myocardium and investigate the expression, regulation and effects of iNOS in this model. Experiments were performed using primary cultures of neonatal rat ventricular myocytes.

A cellular model of hibernating myocardium

Previous models of hibernating myocardium have used whole animals, mostly pigs, with chronic low coronary flow induced by a clamp around the left anterior descending coronary artery. Given that one of the defining features of hibernating myocardium is a reversible downregulation in contractile work in response to low coronary flow, the intrinsic synchronous beating of neonatal rat ventricular myocytes in culture makes this an appropriate choice of model.
A cellular model of hibernating myocardium was established using neonatal rat ventricular myocytes, harvested from neonatal rats aged 1-3 days, subjected to prolonged hypoxia (1% oxygen for 48 hours). In response to hypoxia, it was shown that neonatal rat ventricular myocyte cultures significantly reduced their beating rate (but not the degree of contraction). However this recovered on reoxygenation or with the addition of the isotope, dobutamine.

Experiments were designed to determine the biochemical characteristics of this cellular model of hibernating myocardium. As described for whole animal models, lactate was mildly increased and cellular ATP content was decreased in response to hypoxia.

*Expression of iNOS in a cellular model of hibernating myocardium*

No evidence of iNOS expression was detected in the cellular model of hibernating myocardium although iNOS expression was consistently demonstrated in response to the addition of Interleukin 1β (IL-1β) to the culture medium. The effects of hypoxia on iNOS expression were further investigated using cultures harvested from neonatal rats aged 7 days. In these cultures there was consistent iNOS expression in response to prolonged hypoxia and in response to the addition of IL-1β, but not in the normoxic controls.
Regulation of iNOS expression in response to hypoxia

The role of transcription factors Nuclear Factor kappa B (NFκB) and Hypoxia Inducible Factor 1 (HIF 1) in the expression of iNOS in response to hypoxia were investigated using electromobility shift assays. Nuclear protein binding to both the iNOS NFκB binding site and the iNOS Hypoxia Responsive Element were demonstrated in response to prolonged hypoxia in neonatal rat ventricular myocyte cultures harvested from neonatal rats aged 7 days, suggesting a role for both transcription factors under these hypoxic conditions. However there was no significant upregulation of HIF 1α, compared to normoxic controls, detected on Northern analysis.

Expression of cytokines was investigated by Northern analysis or RT PCR. No IL1β expression was detected, indicating that it has no role in the regulation of iNOS expression under these hypoxic conditions. Expression of Interleukin 6 (IL-6) and Tumour Necrosis Factor α (TNF α) was variable, suggesting a possible contribution of these cytokines to the regulation of iNOS induction under hypoxic conditions.

The effects of iNOS expression in response to hypoxia

The downregulation of contractile work in this cellular model of hibernating myocardium, as evidenced by a reduction in intrinsic beating rate in response to hypoxia, was independent of iNOS activity as no iNOS expression was demonstrated in this model. The effects of iNOS expression under conditions of chronic hypoxia were therefore investigated using neonatal rat ventricular myocyte cultures harvested
from neonatal rats aged 7 days. Increased apoptosis was demonstrated in hypoxic cardiac myocytes when compared to normoxic controls. The role of iNOS in hypoxia induced apoptosis was investigated by the addition of an iNOS inhibitor to the culture medium of cells subjected to hypoxic conditions. Results were confirmed using a non specific NOS inhibitor. iNOS inhibition resulted in a significant reduction in apoptosis of hypoxic myocytes indicating that the mechanism of hypoxia induced apoptosis is in part mediated by iNOS.

However, a number of flasks within each experiment showed absolutely no effect of iNOS inhibition suggesting that other pathways to apoptosis may be activated in certain flasks which may have a lower threshold for commitment to cell death. This is supported by the variable TNF α expression demonstrated in both hypoxic and normoxic cultures. In those cultures expressing TNF α there may be direct stimulation of the DR1 receptor, therefore activating the death receptor pathway to apoptosis.

Conclusions

The results described suggest that iNOS has no role in the protective downregulation of contractile function in hibernating myocardium. In fact the results implicate iNOS expression as a cause of continuous myocyte loss through apoptosis and may be responsible in part for the deterioration from reversible to irreversible cardiac dysfunction with an associated poorer prognosis in patients with hibernating myocardium.