Tolerance and Resistance to Organic Nitrates in Human Blood Vessels

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Table of Contents

Summary vii

Declaration x

Acknowledgments xi

Abbreviations xii

Publications xiii

1. INTRODUCTION

1.1 Historical Perspective 1

1.2 Chemical Structure and Available Preparations 1

1.3 Cellular/biochemical mechanism of action 2

1.3.1 What is the pharmacologically active moiety? 3

1.3.2 How is the active moiety formed? 4

1.3.3 Which enzyme system(s) is involved in nitrate bioconversion? 5

1.3.4 What is the role of sulphydryl groups in nitrate action? 9

1.3.5 Cellular mechanism of action after release of the active moiety. 11

1.4 Pharmacokinetics 12

1.5 Pharmacological Effects 15

1.5.1 Vascular effects 15

1.5.2 Platelet Effects 17

1.5.3 Myocardial effects 18

1.6 Clinical Efficacy 18

1.6.1 Stable angina pectoris 18

1.6.2 Unstable angina pectoris 21

1.6.3 Acute myocardial infarction 21

1.6.4 Congestive Heart Failure 23

1.6.5 Other 24

1.7 Relationship with the endothelium and EDRF 24

1.7.1 EDRF and the endothelium 24

1.7.2 Nitrate-endothelium interactions 27

1.8 Factors limiting nitrate efficacy - Nitrate tolerance 28

1.8.1 Historical notes 28

1.8.2 Clinical evidence for nitrate tolerance 29

1.8.3 True/cellular nitrate tolerance 31

1.8.3.1 Previous studies 31

1.8.3.2 Postulated mechanisms of true/cellular tolerance 33

1.8.3.2.1 The "Sulphydryl depletion" hypothesis 33

1.8.3.2.2 Desensitization of guanylate cyclase 35

1.8.3.2.3 Impaired nitrate bioconversion 36

1.8.3.2.4 The "superoxide hypothesis" 38

1.8.3.2.5 Other possible mechanisms 42

1.8.4 Pseudotolerance 42

1.8.4.1 Neurohumoral activation 42

1.8.4.2 Increased vascular sensitivity to vasoconstrictor agents 43

1.8.4.3 Relationship with true/cellular tolerance 44

1.8.4.4 Clinical implications of pseudotolerance 44

1.9 Factors limiting nitrate efficacy- Nitrate resistance 45

1.9.1 Historical notes 45

1.9.2 Evidence for nitrate resistance 46

1.9.3 Possible mechanisms of nitrate resistance 47

1.10 Scope of present study 48

2. METHODS AND MATERIALS 52

2.1 Methods 52

2.1.1 Subjects 53

2.1.2 Experimental Protocols 53

2.1.2.1 Assessment of Vascular Reactivity 54
2.1.2.2 Measurement of GTN Bioconversion - Tissue GTN/GDN Assay 57
2.1.2.3 Measurement of Vascular Superoxide (O_2^-) Production 58

2.2 Data Analysis **59**

2.3 Materials **59**

3. NITRATE TOLERANCE: MECHANISM(S) OF GTN TOLERANCE INDUCTION IN VIVO **60**

3.1 Introduction **60**

3.2 Experimental Protocol **63**
3.2.1 Vascular Reactivity Studies 63
3.2.2 GTN Bioconversion Studies 64
3.2.3 Vascular O_2^- Generation 64
3.2.4 Data Analysis 64

3.3 Results **65**
3.3.1 Patient Characteristics 65
3.3.2 Vasoconstrictor Responses 65
3.3.3 Vasodilator Responses 66
3.3.4 GTN Bioconversion 67
3.3.5 Vascular O_2^- Generation 67

3.4 Discussion **67**

4. NITRATE TOLERANCE: IN VITRO MODULATION OF GTN EFFECT AND GTN TOLERANCE **92**

4.1 Effect of Diethyldithiocarbamate on GTN Vasorelaxant Responses **92**
4.1.1 Introduction 92
4.1.2 Experimental Protocol 94
4.1.2.1 Effect of DETCA on Vasoconstrictor Responses 95
4.1.2.2 Effect of DETCA on IMA O_2^- Generation and Relaxant Responses to GTN 95

4.1.2.3 Effect of DETCA on GTN Bioconversion 95
4.1.2.4 Data Analysis 96
4.1.3 Results 96
4.1.3.1 Vasoconstrictor Responses 96
4.1.3.2 IMA O_2^- Generation and Relaxant Responses to GTN 96
4.1.3.3 GTN Bioconversion 96
4.1.4 Discussion 97

4.2 Effect of Diphenyleneiodonium Sulfate in GTN Vasorelaxant Responses 104
4.2.1 Introduction 104
4.2.2 Experimental Protocol 106
4.2.2.1 Effect of DPI on GTN Relaxant Responses 106
4.2.2.2 Data Analysis 106
4.2.3 Results 106
4.2.3.1 GTN Relaxant Responses 107
4.2.4 Discussion 107

4.3 Effect of removal of the endothelium on GTN relaxant responses in nontolerant and tolerant human vessels 113
4.3.1 Introduction 113
4.3.2 Experimental Protocol 115
4.3.2.1 Effect of Endothelial Removal on Responses to GTN 115
4.3.2.2 Data Analysis 115
4.3.3 Results 116
4.3.3.1 GTN Relaxant Responses 116
4.3.4 Discussion 116

4.4 Effects of Soluble Guanylate Cyclase Inhibition on Vasodilator Responses to NO Donors in Human Vessels 121
4.4.1 Introduction 121
4.4.2 Experimental Protocol 122
4.4.2.1 Effect of ODQ on Relaxant Responses 123
4.4.2.2 Data Analysis 123
4.4.3 Results 123

4.4.3.1 Effect of ODQ on Relaxant Responses 123
4.4.4 Discussion 124

5. NITRATE CROSS-TOLERANCE: THE RELATIONSHIP OF LONG-TERM ORAL NITRATE THERAPY WITH EX VIVO RESPONSES OF HUMAN VESSELS TO GTN 132

5.1 Introduction 132

5.2 Experimental Protocol 134
5.2.1 Patient Selection 134
5.2.2 Vascular Reactivity Studies 134
5.2.3 Data Analysis 135

5.3 Results 135
5.3.1 Studies in IMA Segments 136
5.3.2 Studies in SV Segments 136

5.4 Discussion 137

6. NITRATE TOLERANCE: EFFECT OF INCREMENTAL SULPHYDRYL BIOAVAILABILITY 146

6.1 Introduction 146

6.2 Experimental Protocol 149
6.2.1 Vascular Reactivity Studies 149
6.2.2 GTN Bioconversion Studies 150
6.2.3 Data Analysis 150

6.3 Results 151
6.3.1 Vascular Reactivity Studies 151
6.3.1.1 Effect of IV NAC on Relaxant Responses to GTN 151
6.3.1.2 Effect of NAC in Vitro on Relaxant Responses to GTN 152
6.3.2 GTN Bioconversion Studies 152

6.4 Discussion 153

7. NITRATE RESISTANCE **170**

7.1 Introduction **170**

7.2 Experimental Protocol **174**

7.2.1 Vascular Reactivity Studies 174

7.2.2 Vascular O_2^- Generation 174

7.2.3 Data Analysis 175

7.3 Results **175**

7.3.1 Determinants of Vascular Responsiveness to GTN 175

7.3.2 Vascular O_2^- Generation 177

7.4 Discussion **177**

8. GENERAL DISCUSSION AND FUTURE DIRECTIONS **198**

8.1 Major limitations and future directions **201**

Summary

1) The major categories of hypotheses of nitrate tolerance are a) impaired nitrate bioconversion resulting in diminished nitric oxide (NO) release b) increased NO clearance mediated via increased generation of superoxide (O_2^-) and/or c) desensitization of guanylate cyclase. The supporting evidence for these studies is based largely on animal studies.

2) The primary aim of the experiments described in this thesis was to investigate the mechanism(s) of nitrate tolerance) in vessels from patients receiving a 24 hour intravenous infusion of glyceryl trinitrate (GTN) at 10 μg/min. The vessels studied were isolated segments of internal mammary artery (IMA) and saphenous vein (SV) obtained from patients undergoing coronary bypass surgery.

3) Pharmacological studies were performed in organ baths with the vessels mounted under tension (2g for IMA; 1g for SV).

4) Responses to GTN were reduced 3- to 5-fold in the vessels from the nitrate group compared with vessels from control patients, demonstrating induction of a moderate degree of tolerance with this GTN regimen.

5) Tolerance was associated with minimal cross-tolerance to sodium nitroprusside (SNP) and A23187, indicting that tolerance was nitrate-specific. Other studies confirmed that GTN and SNP vasodilator action are mediated via guanylate cyclase in human vessels. The lack of significant cross-tolerance was therefore consistent with the impaired bioconversion hypothesis

6) This finding was supported by measuring bioconversion of GTN to 1,2-glyceryl dinitrate (1,2-GDN) following brief exposure to GTN. The concentration of 1,2-GDN was lower in segments of SV from the nitrate group compared with segments from the control group, indicating impairment of the bioconversion process.

7) With regard to the O_2^- hypothesis, IMA O_2^- generation was found to be greater in segments from the nitrate group than from those in the control group. However, inhibition of superoxide dismutase in vitro, which produced a 3-fold increase in IMA O_2^- generation, did not affect responses to GTN or bioconversion of GTN. These results suggest GTN action is insensitive to acute increases in redox stress.

8) Removal of the endothelium did not affect GTN responsiveness in IMA segments from either control or tolerant patients, suggesting that tolerance induction is independent of the endothelium.

9) Diphenyleneiodonium (DPI) was found to have only a small inhibitory effect on the relaxant responses of IMA to GTN in control segments, suggesting that the cyctochrome-P450 enzyme system play a minor role in GTN-induced vasodilation in human vessels.

10) Co-infusion of intravenous N-acetylcysteine (NAC) at 10 g/24 hours with GTN had minimal effect on the vasodilator responses to GTN and bioconversion of GTN. Similarly, exposure of tolerant and non-tolerant vessels to NAC in vitro did not affect responsiveness to GTN. These results provide evidence that NAC does not prevent or reverse GTN tolerance in these human vessels.

11) Vessels obtained from patients receiving prophylactic oral nitrate therapy (60-120 mg ISMN-SR once-daily or 10-20 mg ISDN thrice-daily) exhibited cross-tolerance to GTN. A secondary finding was that tolerance to GTN was seen up to 29 hours following the last dose of ISMN-SR, whereas there was no evidence of tolerance 17 hours after the last dose of ISDN..

12) The availability of control data permitted evaluation of the determinants of de novo vasodilator responses to GTN. Hyporesponsiveness to GTN (GTN resistance) was observed in the IMA but not SV, and was associated with increasing total number of risk factors for coronary artery disease and specifically with prior hypercholesterolaemia, smoking or diabetes mellitus. In addition, a correlation was

found between IMA responses to GTN and responses to A23187, a conventional marker of endothelial function.

13) Prior hypercholesterolaemia was also associated with higher levels of O_2^- generation. These experiments demonstrate that the presence of some risk factors for coronary artery disease may impair responses of arterial smooth muscle to GTN. Furthermore, the results suggest that the mechanism underlying this "resistance" to GTN may be related to increased redox stress.