Mechanisms Underlying Mesenteric Ischemia/Reperfusion Induced Intestinal Dysmotility

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Abstract

**Background and aims:** Mesenteric ischemia-reperfusion (I/R) is a severe vascular emergency, associated with poor prognosis and relatively high morbidity and mortality, which requires early diagnosis and intervention. On the contrary to numerous studies on I/R induced microvascular changes, very few studies are available on the impact of mesenteric I/R on intestinal dysmotility. The present study therefore aimed at investigating time course of changes in intestinal smooth muscle responsiveness and the roles of inducible cyclooxygenase (COX) and nitric oxide synthase (iNOS) and reactive oxygen species (ROS) in mesenteric I/R-induced dysmotility thus to establish an intrinsic links among these factors that could eventually lead to set up a scientifically reliable screening platform for drugs with potential therapeutic values for the treatment of ischemia-associated gut motor dysfunction.

**Methods:** C57BL/6J mice, iNOS knockout, and COX-2 knockout mice were used in this study. Mesenteric I/R was performed in mice by clamping the superior mesenteric artery for 30 min followed by reperfusion with different time periods respectively. Intestinal smooth muscle strips were mounted in organ baths filled with oxygenated Krebs solution, and the isometric tensions developed in response to various agonists and inhibitors were recorded. The releases of prostanoids were measured by ELISA kits. The levels of protein expressions in ileum were detected by Western blotting. Immunofluorescent staining was performed to show the cellular localizations of the proteins. The production of reactive oxygen species (ROS) were also measured by electron paramagnetic resonance spectroscopy (EPR) and spin trap.
**Results:** After 4-hr reperfusion, the contractions of longitudinal smooth muscle strips to acetylcholine (ACh, $10^{-8}$ M to $3 \times 10^{-5}$ M), histamine ($10^{-8}$ to $10^{-4}$ M) were reduced, while the responses to serotonin (5-HT $10^{-8}$ to $10^{-5}$ M), U46619 ($10^{-9}$ to $10^{-6}$ M), and substance P ($10^{-7}$ M) were unaffected. The contractions of longitudinal smooth muscle strips to serotonin, ACh, and U46619 were enhanced after 24-hr reperfusion. The reduced spontaneous contractility and contractile responses to carbachol (CCh) in the circular muscle layer was restored by an acute treatment of non-specific nitric oxide synthase (NOS) inhibitor (L-NAME) and selective inducible NOS inhibitor, AMT, cyclooxygenase-2 (COX-2) inhibitor celecoxib, and prostacyclin receptor antagonist CAY10441 in I/R injured tissue. In iNOS knockout and COX-2 knockout homozygous (-/-) mice, the inhibitory effect of I/R on gut smooth muscle contractility was greatly attenuated.

The results with measurement of prostanoids strongly indicated that prostaglandins PGI$_2$ and PGE$_2$ are likely to be responsible for the loss of smooth muscle contractility after mesenteric I/R since their productions were inhibited by celecoxib and also AMT. Western blotting analysis showed significant up-regulations of COX-2, iNOS, nitrotyrosine, NF-κB protein expressions, which were mainly confined to the muscle layers of mouse intestine after mesenteric I/R injury with no obvious changes in the expression of COX-1 and p47$^{phox}$ NAPDH oxidase, but nNOS was found to be down-regulated. Immunostaining results further revealed that mesenteric I/R increased the iNOS immunoreactivity in the resident macrophages as confirmed by macrophage marker F4/80. iNOS was also expressed in myenteric nervous networks, infiltrating cells, and
smooth muscle cells, while COX-2 were expressed mainly in the myenteric nerves and infiltrating cells.

Increased production of ROS in response to I/R was detected by EPR spin trap method. Increased ROS formation was observed after mesenteric I/R in wild type, iNOS and COX-2 knockout mice, which can be attenuated by ROS scavenger Tiron and Detca but not by inhibitors of iNOS or COX-2.

**Conclusion:** In summary, the present study has provided useful information regarding on cellular cascade leading impaired gut motility following mesenteric I/R insult and the cellular signaling molecules involved include ROS, iNOS and COX-2, thus providing possible therapeutic targets for the treatments of inflammation-associated with gut dysmotility.
Table of Contents

Declaration i
Abstract ii
Acknowledgements v
Table of Contents vi
List of Figures viii
List of Tables x
Abbreviations xi

Chapter I Introduction

1.1 Background of Mesenteric Ischemia/Reperfusion (I/R).................................1
  1.1.1 Clinical Relevance of Mesenteric I/R.................................................1
  1.1.2 Cellular and Molecular Responses after Mesenteric I/R.........................1
  1.1.3 Current Research Progress and Interest.............................................3
    1.1.3.1 Microvascular Changes.............................................................3
    1.1.3.2 Nuclear Receptors.......................................................................5
    1.1.3.3 Apoptotic Changes.....................................................................6
  1.1.4 Pharmacological Interventions............................................................6
    1.1.4.1 Protective Natural Products and Nutrients against Mesenteric I/R.......7
  1.1.5 Ischemic Preconditioning (IPC)...........................................................9
  1.1.6 Effect of Mesenteric I/R on Intestinal Motility.....................................9
1.2 Role of Nitric Oxide in the GI Tract..........................................................10
  1.2.1 Physiological Functions of Nitric Oxide Synthase (NOS) Isoforms..........10
  1.2.2 Characteristics of Inducible Nitric Oxide Synthase (iNOS)..................11
  1.2.3 eNOS and nNOS in Gastrointestinal Diseases.....................................12
  1.2.4 Involvement of iNOS in Mesenteric I/R.............................................13
  1.2.5 Involvement of iNOS in Other Bowel Diseases..................................15
1.3 Nitric Oxide and Oxidative Stress...........................................................16
1.4 Cyclooxygenases and Prostaglandins in the GI Tract................................17
1.5 Objectives.................................................................................................18

Chapter II Materials and Methods...............................................................19

2.1 Animals......................................................................................................19
2.2 Surgical Procedures...................................................................................19
2.3 Tissue Preparation....................................................................................20
2.4 Experimental Protocols for Functional Studies..........................................23
2.5 Western Blotting.......................................................................................25
2.6 Whole Mount Preparation and Immunohistochemistry...............................26
2.7 Electron Paramagnetic Resonance (EPR) Spectroscopy and Spin Trap.........27
2.8 Drugs, Chemicals and Solutions..............................................................28
2.8.1 Composition of Krebs Solution ............................................................... 31
2.8.2 Solutions for Western Blotting ................................................................. 31
2.9 Antibodies Used in Western Blotting and Immunohistochemistry ............. 34
2.10 Data Analysis ............................................................................................... 35

Chapter III Results .............................................................................................. 36

3.1 Changes of Intestinal Smooth Muscle Responsiveness after Mesenteric I/R .................................................................................................................. 36
3.2 Functional Inhibition of Kinetically Active Mediators .................................. 44
3.3 Genetic Modulation of NOSII and COX-2 Function in Knockout Animals .... 56
3.4 Prostaglandin Production by I/R Injured Intestinal Tissue ............................. 60
3.5 Expression of iNOS and COX-2 in the Muscularis Layer of Intestine ............ 63
3.6 Localization Study of the Intestinal Muscularis after Mesenteric I/R ............ 73
3.7 Involvement of Reactive Oxygen Species (ROS) during Mesenteric I/R Injury 84

Chapter IV Discussion ......................................................................................... 91

Chapter V General Conclusion ........................................................................... 110

References............................................................................................................ 112

Curriculum Vitae ................................................................................................... 121