IMPAIRED TISSUE RESPONSIVENESS TO B-TYPE NATRIURETIC PEPTIDE IN HEART FAILURE: BIOCHEMICAL BASES

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Table of contents

Abstract

Release of the B-type natriuretic peptide (BNP) is increased in heart failure (HF). Physiologically, BNP exerts natriuretic, diuretic, vasodilator and cardioprotective effects. A number of clinical investigations carried out with synthetic BNP for the treatment of HF have suggested that BNP-based restoration of homeostasis is inadequate. This equivocal clinical benefit of a recombinant BNP in HF raises the possibility of attenuated BNP response.

The objective of this thesis is an examination of three aspects of BNP-related cardiovascular pathophysiology. The first issue tested was the effect of BNP in isolated neutrophils of control subjects via neutrophil superoxide (O_2^-) generation. The second issue was integrity of BNP effects in acute and chronic HF patients, and the third was maintenance of BNP effect in the acute phase of Tako-tsubo cardiomyopathy (TTC), a form of "stress-induced" cardiomyopathy and another condition associated with increased BNP release.

The study utilized the technique of electron paramagnetic resonance spectroscopy to quantitate the extent of O_2^- generation from isolated neutrophils. In control subjects, the data showed significant suppression of O_2^- generation in PMA- and fMLP-stimulated neutrophils. This effect was not affected by either age or gender of the study population. The effect of BNP was mimicked by a cell-permeable cGMP analog (8-pCPT-cGMP) and partially restored by a protein kinase G (PKG) inhibitor KT5823. Furthermore, BNP inhibited the fMLP-induced phosphorylation of the NAD(P)H oxidase subunit p47phox at ser345. These findings led to the conclusion that BNP suppression of O_2^- generation is mediated by the cGMP-PKG signaling pathway.

The studies concerning HF patients had two major components: (a) examination of the BNP effect in acute HF patients and (b) determination of changes in effect with chronic treatment of such patients.

Studies with acute HF patients showed a significant attenuation of BNP effects on suppressing neutrophil O_2^- generation compared with control subjects. However, 8-p-CPT-cGMP effects were retained, which indicates that BNP effects were attenuated at the level of natriuretic peptide receptor level. Furthermore, the effect of BNP on inhibition of the fMLP-induced phosphorylation of p47phox at ser345 was lost in acute HF patients. Comparison of the acute and chronic HF patients revealed a partial restoration of BNP effects, especially in younger patients.

TTC is associated with acute release of BNP into plasma as a result of inflammatory change in the heart. It was found that BNP effect was attenuated similarly in TTC patients and acute HF patients. The residual effect was not associated with either patients' inflammatory status or catecholamine release.

In summary, this study identified that (1) In control subjects without diagnosed cardiovascular disease, BNP suppressed the release of the inflammatory modulator O_2^- from isolated neutrophils by attenuating NAD(P)H oxidase assembly; (2) This effect of BNP was lost in patients with acute HF, but recovers partially with chronic treatment of HF. (3) In TTC patients, attenuation of BNP effect was also present.

These data suggest that marked elevation of plasma concentration of BNP limits its physiological anti-inflammatory effects.

Declaration

I, Saifei Liu, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Publications, presentations and awards related to the work conducted towards this thesis

Publications related to the work conducted in this thesis:

Liu, S, Ngo, DT, Stewart, S, Horowitz, JD & Chirkov, YY 2014 BNP suppression of neutrophil superoxide generation: mechanistic studies in normal subjects. Clin Exp Pharmacol Physiol. No. 41 Aug 12, pp 739-743.

Liu, S, Ngo, DT, Chong, CR, Amarasekera, AT, Procter, NE, Licari, G, Dautov, RF, Stewart, S, Chirkov, YY & Horowitz, JD 2015, 'Suppression of neutrophil superoxide generation by BNP is attenuated in acute heart failure: a case for 'BNP resistance'', Eur J Heart Fail, Feb 11. doi:10.1002/ejhf.242

Presentations of work related to this thesis on international conferences:

Liu, S, Ngo, DT, Chirkov, YY, Sheikh, AR, Licari, G, Stewart, S & Horowitz, JD. Brain Natriuretic Peptide (BNP) Suppresses Neutrophil Superoxide Release: Attenuation in Patients with Congestive Heart Failure (CHF). Presented at AHA scientific sessions 2012, Los Angeles, USA. *Circulation, vol. 126. A17067.*

Liu, S, Ngo, DT, Chirkov, YY, Stewart, S & Horowitz, JD. Impaired B-type Natriuretic Peptide Effets on Neutrophil Burst in Heart Failure Patients. Presented at AHA scientific sessions 2013, Dallas, USA. *Circulation, vol. 128. A13335.*

Liu, S, Ngo, DT, Chong, CR, Chirkov, YY, Stewart, S & Horowitz, JD. Mechanisms of BNP interactions with neutrophil superoxide release: attenuation in acute and chronic heart failure. Presented at World Congress of Cardiology 2014, Melbourne, Australia. *Global Heart Journal 2014; 9: 1S: O066.*

Liu, S, Ngo, DT, Stansborough, J, Raman, B, Chirkov, YY, Horowitz, JD. Rapid desensitization of neutrophils to anti-inflammatory effects of BNP: implications in Tako-tsubo cardiomyopathy. Presented at International Society of Cardiovascular Pharmacotherapy (ISCP) 2014, Adelaide, Australia.

Liu, S, Ngo, DT, Chan, WP, Chirkov, YY, Horowitz, JD. Tachyphylaxis to the anti-inflammatory effects of BNP in acute heart failure:-potential response to therapy. Heart Failure 2015 / 2nd World Congress on Acute Heart Failure. Seville, Spain.

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List of abbreviations

Abbreviation	Term
8-pCPT-cGMP	8-(4-Chlorophenylthio)-guanosine 3′,5′-cyclic monophosphate
ACE	Angiotensin converting enzyme
ADMA	Asymmetric dimethylarginine
ADP	Adenosine diphosphate
AIx	Augmentation index
AngII	Angiotensin II
ANP	Atrial natriuretic peptide
ApoA-I	Apolipoprotein A-I
ASCEND-HF trial	Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure trial
AVP	Arginine vasopressin
BH4	Tetrahydrobiopterin
BNP	B-type natriuretic peptide
CAD	Coronary artery disease
CAT1-H	1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl-trimethylammonium chloride

cGMP	cGMP cyclic 3',5'- guanosine monophosphate
CGN	cGMP-gated ion channels
CGRP	Calcitonin gene-related peptide
CM-H	1- hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl pyrrolidine
CNP	C-tpye natriuretic peptide
CP-H	1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine hydrochloride
CRP	C-reactive protein
Cyto B	Cytochalasin B
db-cGMP	N2,2'-O-dibutyryl guanosine 3':5'-cyclic monophosphate
DMSO	Dimethyl sulfoxide
DPI	Diphenyleneiodonium
DPPIV	Dipeptidyl peptidase IV
EMPO	2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide
eNOS	Endothelial nitric oxide synthase
EPR	Electron paramagnetic resonance
ESR	Electron spin resonance
ET-1	Endothelin-1

fMLP	*N*-formyl-methionyl-leucyl-phenylalanine
GM-CSF	Granulocyte/macrophage colony stimulating factor
GPx	Glutathione peroxidase
HBSS	Hanks' balanced salt solution
HCl	Hydrochloric acid
HDL	High-density lipoprotein
HF	Heart failure
H_2O_2	Hydrogen peroxide
HOCl/HClO	Hypochlorous acid
hs-CRP	High-sensitivity CRP
IBMX	3-Isobutyl-1-methylxanthine
IDE	Insulin-degrading enzyme
iNOS	Inducible nitric oxide synthase
IL	Interleukin
LAMs	Lipoarabinomannans
L-NMMA	N-monomethyl-L-arginine
LPS	Lipopolysaccharide
LTB4	Leukotriene B4

LV	Left ventricular
LVEF	Left ventricular ejection fraction
MCP	Monocyte chemoattractant peptide
MI	Myocardial infarction
mitoTEMPO-H	1-hydroxy-4-[2-(triphenylphosphonio)-acetamido]-2,2,6,6-tetramethylpiperidine
Mn-SOD	Manganese superoxide dismutase
MPO	Myeloperoxidase
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NEP	Neutral endopeptidase
NF	Nuclear factor
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO_2^-	Nitrite
NOS	Nitric oxide synthase
NPs	Natriuretic peptides
NPR-A	Natriuretic peptide receptor-A
NPR-B	Natriuretic peptide receptor-B

NPR-C	Natriuretic peptide receptor-C
NTG	Nitroglycerin
NT-proBNP	N-terminal pro B-type natriuretic peptide
NYHA class	New York heart association class
O_2^-	Superoxide
$OH^·$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
Ox-LDL	Oxidized low density lipoprotein
PAF	Platelet activating factor
PCWP	pulmonary capillary wedge pressure
pGC	Particulate guanylyl cyclase
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PGF2α	Prostaglandin F2α
PGI2	Prostacyclin
PGs	Prostaglandins
PKC	Protein kinase C
PKGs	cGMP-dependent protein kinases

PMA	Phorbol myristate acetate
PP-H	1-hydroxy-4-phosphono-oxy-2,2,6,6-tetramethylpiperidine
Prx-3	Peroxiredoxin-3
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SDMA	Symmetric dimethylarginine
Ser	Serine
sGC	Soluble guanylyl cyclase
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
TLR	Toll-like receptors
TM-H	1-hydroxy-4-methoxy-2,2,6,6-tetramethylpiperidine
TMT-H	*N*-(1-Hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-2-methylpropanamide
TNF-α	Tumor necrosis factor-α
TTC	Takotsubo cardiomyopathy
XO	Xanthine oxidase

List of figures:

List of tables: