Tri-iodothyronine (T3) Therapy in a Pre-Clinical Model of Septic Shock

A thesis submitted for the degree of Doctor of Philosophy

Discipline of Acute Care Medicine
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23rd March 2015

Abstract

Thyroid hormone is essential for normal organ function. Tri-iodothyronine (T3) is the most active form of thyroid hormone, derived from the deiodination of the more abundant thyroxine (T4). T3 is considered to have prominent haemodynamic and metabolic effects.

During illness, blood levels of T3 decline with a reciprocal increase of the inactive reverse-T3 and eventually, a fall of T4. This phenomenon is referred to as Non-Thyroidal Illness Syndrome (NTIS) and the extent of change in circulating thyroid hormones is proportional to severity of disease and survival.

NTIS is particularly marked during sepsis. Sepsis is the most common diagnosis of patients requiring emergency admission to an Intensive Care Unit (ICU) and mortality rates remain high despite provision of all supportive therapies. Given the importance of T3 for normal function and the relationship between low T3 and poor outcome, NTIS may contribute to the multi-organ dysfunction of sepsis.

Restoring T3 levels during sepsis may be beneficial but has received little attention. Concerns that NTIS may be an adaptive response and that T3 supplementation may provoke thyrotoxicity have limited the conduct of clinical trials in patients with septic shock. There is also uncertainty regarding the need to co-administer hydrocortisone (HC) with T3. Consequently, a pre-clinical study was required to test the safety and efficacy of T3 therapy with and without HC.

An ovine model of septic shock was developed, applying many of the supportive care elements provided to humans in an ICU. Following a bolus of intravenous *E.coli*, sheep received 24 hours of protocol-guided sedation, ventilation, parenteral fluids and noradrenaline (NorA) infusion. The model was validated over time and replicated much of the human septic response, including NTIS.

Following pharmaceutical and dose finding studies, a randomised, blinded, placebo-controlled trial of T3 with and without HC, was conducted in the ovine model. After two hours of sepsis, 32 sheep received a 24-hour infusion of:

(i) T3 + placebo, (ii) HC + placebo, (iii) T3 + HC, or (iv) placebo + placebo. The primary outcome was the total amount of NorA required during the infusion of study drugs; while the secondary outcomes included haemodynamic, metabolic and parameters of organ function.

Plasma T3 levels fell in placebo animals and were increased to supra-physiological concentrations by T3 infusion. The amount of NorA required was no different between the study groups (mean ± SEM μg/kg; T3 group, 501 ± 131; T3 + HC group, 466 ± 175; HC group, 167 ± 101; placebo group, 208 ± 160; p = 0.20). There was no significant treatment effect on any haemodynamic variable, temperature, pH, lactate or oxygen extraction.

The same dose of T3 was subsequently tested in a group of non-septic sheep. Despite supra-physiological plasma levels, there was no change to any physiological endpoint.

In conclusion, a 24-hour infusion of T3 (with or without HC) in an ovine model of septic shock did not reduce NorA requirements nor alter any other measured physiological variable. Acute T3 replacement appears to be safe, but the role of this therapy for intractable septic shock remains uncertain.

Declaration

I 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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Acknowledgments

This was a complex study, the likes of which had not been previously conducted in our institution. To start this project, let alone see it to completion required the support of many people from a range of organisations.

My “team” of supervisors, Professor Guy Ludbrook (Acute Care Medicine, University of Adelaide), Professor Marianne Chapman (Acute Care Medicine, University of Adelaide), Professor David Torpy (Department of Medicine, Royal Adelaide Hospital), Professor Iain Clarke (Department of Physiology, Monash University) and Dr Tim Kuchel (Pre-clinical, Imaging and Research Laboratories, South Australian Health and Medical Research Institute) have provided expert guidance, wisdom and understanding. From our conversations, be it in person or remotely, I have learnt much about the importance of robust science and “efficient thinking”. There were many potential obstacles in conducting this study, but their encouragement to “make this work” ensured these were not insurmountable.

Associate Professor Robert Young (Royal Adelaide Hospital, Intensive Care Unit) encouraged and supported me to undertake this project. His emphasis on fostering research has significantly contributed to the calibre of the Intensive Care Unit (ICU) at the Royal Adelaide Hospital.

Dr Jennifer Ong (Acute Care Medicine, University of Adelaide) provided efficient and insightful assistance to ensure this project was completed and conducted according to the University’s requirements.

The animal model required many resources and expertise. Dr Tim Kuchel was integral in advising how to develop a large animal model of disease. He also may have turned me into a “gentleman physiologist” during our chats while performing surgery on over 60 sheep. Dr Susan Porter, Loren Matthews and Melissa Gourlay ensured laboratory resources were available and were always able to lend a pair of expert hands. Dr John Finnie (Veterinary Services Division, Institute of Medical and Veterinary Sciences) provided review of tissue histopathology during model development.

I am grateful to Philips for provided monitoring devices, Edwards Lifesciences for loaning a continuous cardiac output monitor and Baxter for infusion pumps.

Dr Ivan Bastian, Lance Mickan and Paul Sideris (Infectious Disease Laboratory, Institute of Medical and Veterinary Sciences) helped prepare solutions of *E.coli*. Tragically, Lance died in October 2011 and I trust this thesis suitably honours his contribution to medical science.

Each animal study took two days to complete and with constant staffing required, was only possible with the assistance from suitably experienced nursing and medical staff. I am extremely grateful for the ICU nursing expertise and reliability of Jason Edwards, Alex Poole, Alison Ankor and Luke Chester. Drs Jonathan Fraser and Faith Crichton (ICU registrars, Royal Adelaide Hospital) also provided staffing assistance in the sheep laboratory. I trust this was a worthwhile and educational experience for all involved. Stephanie O'Connor (ICU Research Manager, Royal Adelaide Hospital) oversaw the project budget and facilitated availability of nursing staff.

Associate Professor Robert Milne and Courtney Trainor (School of Pharmacy and Medical Science, University of South Australia) provided advice and assistance with the preparation and analysis of T3 in solution. Study drug preparation, trial randomisation and allocation concealment were expertly overseen by Peter Slobodian (Pharmacist, Investigational Drugs, Royal Adelaide Hospital) and performed by pharmacy staff Valerie Sim, Cuong Tang, Melissa Teo, Sally Michail, YingShi Chan and Yee Chai.

Alix Rao (Department of Physiology, Monash University) assisted with hormonal assay of sheep plasma. Tom Sullivan (Discipline of Public Health, University of Adelaide) provided statistical advice.

An ICU model of disease is very expensive. Funding was sought from many organisations, but the fledgling nature of this study did not initially appeal to traditional funding sources. I was subsequently most fortunate to receive financial support from Ms Deidre Tidswell, a local benefactor who generously provided funds to ensure this study could commence. Thereafter, successful application for

partial funding was obtained from the Royal Adelaide Hospital Research Committee (New Investigator Clinical Project Grant, 2011).

Finally, none of this would have been possible without the endless support of my wife, Coralie. Not only did she ensure I had every opportunity to complete this project, but she helped organise study logistics, maintained laboratory equipment and provided ICU nursing expertise … as well as running a household with five children (Liam, James, Rosie, Tom, Matty) and a husband who love her very much.

"If a job is worth doing, it is worth doing well."

Lois Maiden (my grandmother)

Table of Contents

Table of Tables

Table of Figures

Abbreviations

ABG	Arterial Blood Gas
ADP	Adenosine Phosphate
Adr	Adrenaline
AEC	Animal Ethics Committee
AF	Atrial Fibrillation
AG	Anion Gap
AKI	Acute Kidney Injury
ALI	Acute Lung Injury
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMI	Acute Myocardial Infarction
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
APACHE	Acute Physiology and Chronic Health Evaluation
aPTT	Activated Partial Thromboplastin Time
ATN	Acute Tubular Necrosis
ATP	Adenine Tri-Phosphate
Bili	Bilirubin
BMR	Basal Metabolic Rate
BNP	Brain Natriuretic Peptide
CABG	Coronary Artery Bypass Graft
cAMP	3',5'-Cyclic Adenosine-Monophosphate
CI	Cardiac Index
CFU	Colony Forming Units
CLP	Caecal Ligation and Puncture
CMIA	Chemiluminescent Microparticle Immuno-Assay
CNS	Central Nervous System
CPB	Cardio-Pulmonary Bypass
CV	Coefficient of Variation
CVC	Central Venous Catheter
CVP	Central Venous Pressure
D1	Type 1 Deiodinase
D2	Type 2 Deiodinase

D3	Type 3 Deiodinase
DA	Dopamine
DIT	Di-Iodotyrosine
DITPA	3,5-Di-Iodothyropropionic Acid
ED	Emergency Department
EDTA	K3-Ethylene-Diamine-Tetra-Acetic Acid
EF	Ejection Fraction
EGDT	Early Goal Directed Therapy
ELISA	Enzyme-Linked Immunosorbent Assay
FE-Na^+	Fractional Excretion of Sodium
FiO_2	Fraction of Inspired Oxygen
GH	Growth Hormone
GHRH	Growth Hormone Releasing Hormone
H&E	Haematoxylin & Eosin
Hb	Haemoglobin
HC	Hydrocortisone
HPT	Hypothalamo-Pituitary-Thyroid
HR	Heart Rate
ICU	Intensive Care Unit
IQR	Interquartile Range
IFN-γ	Interferon γ
IL	Interleukin
i.p.	Intra-peritoneal
IP3	Inositol-1,4,5-Triphospahte
i.v.	Intravenous
K_M	Michaelis Constant
KO	Knock Out
LV	Left Ventricle
LVSWI	Left Ventricular Stroke Work Index
MAb	Monoclonal Antibody
MAP	Mean Arterial Pressure
MCT	Mono-Carboxylate Transporters
MIT	Mono-Iodotyrosine
mPAP	Mean Pulmonary Artery Pressure
mRNA	Messenger Ribonucleic Acid

mt-DNA	Mitochondrial Deoxyribo-Nucleic Acid
MW	Molecular Weight
NADH	Nicotinamide Adenine Di-Nucleotide
NO	Nitric Oxide
NorA	Noradrenaline
NTCP	Na^+ / Taurocholate Co-transporting Polypeptide
NTIS	Non-Thyroidal Illness Syndrome
OATP	Organic Anion Transporting Polypeptides
OER	Oxygen Extraction Ratio
PA	Pulmonary Artery
$PaCO_2$	Partial Pressure of Carbon Dioxide in Arterial Blood
PAdP	Pulmonary Artery Diastolic Pressure
PaO_2	Partial Pressure of Oxygen in Arterial Blood
PAP	Pulmonary Artery Pressure
PBI	Protein Bound Iodine
Pbo	Placebo
PCV	Packed Cell Volume
PEEP	Positive End Expiratory Pressure
PLT	Platelet
PMCA	Plasma Membrane Calcium-ATPase
PRL	Prolactin
PT	Prothrombin Time
PTU	Propylthiouracil
PVRI	Pulmonary Vascular Resistance Index
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RIA	Radio-Immuno-Assay
rT3	Reverse Tri-iodothyronine
RVSWI	Right Ventricular Stroke Work Index
SaO_2	Oxygen Saturation of Haemoglobin in Arterial Blood
s.c.	Subcutaneous
SD	Standard Deviation
SERCA	Sarcoplasmic Reticulum Calcium-ATPase
SIMV	Synchronised Intermittent Mandatory Ventilation
SIRS	Systemic Inflammatory Response Syndrome

SMR	Standardised Mortality Ratio
SpO_2	Pulse Oxygen Haemoglobin Saturation
SR	Sarcoplasmic Reticulum
SvO_2	Oxygen Saturation of Haemoglobin in Venous Blood
SVR	Systemic Vascular Resistance
SVRI	Systemic Vascular Resistance Index
$t_{1/2}$	Half-life
T3	Tri-iodothyronine
T3S	Sulphated Tri-iodothyronine
T4	Thyroxine
T4S	Sulphated Thyroxine
TBG	Thyroid Hormone Binding Globulin
Tg	Thyroglobulin
TNF-α	Tissue Necrosis Factor-α
TPO	Thyroid Peroxidase
TR	Thyroid Hormone Nuclear Receptors
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone (Thyrotropin)
TTR	Transthyretin
VBG	Venous Blood Gas
VO_2	Oxygen Consumption
V_D	Volume of Distribution
V_T	Tidal Volume
WCC	White Cell Count

Physiological Equations

Cardiovascular

Systemic Vascular Resistance Index (SVRI)

(MAP – Right Atrial Pressure) / CI [x 79.9] = dyn.s / $cm^5.m^2$

CVP was used as an estimate of right atrial pressure

Pulmonary Vascular Resistance Index (PVRI)

(mPAP – Left Atrial Pressure) / CI [x 79.9] = dyn.s / $cm^5.m^2$

Pulmonary artery diastolic pressure (PAdP) was used as an estimate of left atrial pressure

Left Ventricular Stroke Work Index (LVSWI)

Stroke Volume Index x MAP x 0.0144 = g.m / m^2

Right Ventricular Stroke Work Index (RVSWI)

Stroke Volume Index x mPAP x 0.0144 = g.m / m^2

Respiratory

P:F

PaO_2 (mmHg) : FiO_2

Minute Ventilation

Tidal Volume (V_T) x Ventilation Rate = mL / minute

Pulmonary Compliance

Tidal Volume (V_T) / Plateau Inspiratory Pressure = mL / cmH_2O

Renal

Creatinine Clearance

[Urine Creatinine concentration (mmol/L) x Urine flow rate (mL/min)] /

Serum creatinine concentration (mmol/L) = mL/min

Fractional Excretion Na^+

[Urine Na^+ concentration (mmol/L) x Serum Creatinine concentration (mmol/L)] /

[Plasma Na^+ concentration (mmol/L) x Urine Creatinine concentration (mmol/L)]

Metabolic

O_2 Delivery (DO_2) Index

$[1.39 \times Hb\ (g/L) \times SaO_2 + (0.003 \times PaO_2)] \times CI = mL / min / m^2$

O_2 Consumption (VO_2) Index

$[1.39 \times Hb \times (SaO_2 - SvO_2)] \times CI = mL / min / m^2$

O_2 Extraction Ratio

$(SaO_2 - SvO_2) / SaO_2$

Anion Gap

$[Na^+ + K^+] - [Cl^- + HCO_3^-]$

Thyroid Hormone Concentration Conversion from Traditional Units to International System (SI) of Units.

	Human Normal Range (SI Units)	**Human Normal Range** (Traditional Units)	**Convert from Traditional to SI**
Total T3	1.2 – 2.7 nmol/L	80 – 200 ng/dL 0.8 – 2.0 ng/mL	x 0.015 x 1.536
Free T3	3.5 – 6.1 pmol/L	2.3 – 4.2 pg/mL	x 1.536
Total rT3	0.22 – 0.46 nmol/L	14 – 30 ng/dL 0.14 – 0.3 ng/mL	x 0.0154 x 1.536
Total T4	58 – 160 nmol/L	4.5 – 12.5 μg/dL 45 – 125 ng/mL	x 12.87 x 1.287
Free T4	10 – 23 pmol/L	0.8 – 1.8 ng/dL 80 – 180 ng/mL	x 12.87 x 0.1287

Thesis Overview

Chapter 1: Thyroid Hormone

An understanding of the normal physiology of the thyroid axis is required to appreciate the changes that occur to thyroid hormones during illness. This chapter describes the thyroid hormones, their synthesis, kinetics, effect on the cell and each organ system.

Chapter 2: Thyroid Hormone Changes and Treatment During Non-Thyroidal Illness

This chapter outlines the changes to thyroid hormones for a range of diseases, the likely mechanisms for these disturbances and summarises the studies investigating the effect of thyroid hormone replacement in non-thyroidal illness. The controversy of T3 replacement in critical illness is discussed and the case made for a pre-clinical trial in septic shock.

Chapter 3: Development and Validation of an Ovine Model of Septic Shock

Following a discussion on the limitations of previous animal models of sepsis, this chapter will outline the development and validation of an ovine model that replicates typical features of septic shock and incorporates many elements of the supportive care provided to a septic human in ICU. This model will be used to test the effect of T3 replacement.

Chapter 4: T3 Pharmacology

A systematic review of previous T3 studies was undertaken to determine doses used, plasma levels achieved and endpoints measured. A pharmaceutical study was performed to ensure stability of T3 in solution and compatibility with administering equipment. Pilot studies were then conducted to determine the dose of T3 that should be tested in septic sheep.

Chapter 5: Tri-iodothyronine Administration, with and without Hydrocortisone, in an Ovine Model of Septic Shock

This chapter outlines a randomised, blinded, placebo-controlled trial of T3, with and without HC, in the ovine model of septic shock. Hormonal therapy increased plasma T3 concentrations but did not alter the primary endpoint (noradrenaline dose) or any other physiological parameter. Possible reasons for the lack of experimental effect and validity of the study are discussed.

Chapter 6: Tri-iodothyronine in Non-septic Sheep

The same dose of T3 used in the sepsis study was tested in non-septic sheep. Plasma concentrations of T3 were higher in non-septic animals but again were not associated with any physiological changes over 24 hours. The effect of sepsis on plasma T3 levels are examined.

Chapter 7: Future Studies

A number of other research questions became apparent during the conduct of this thesis. Further projects are proposed to explore observations noted during development of the sepsis model, investigate the mechanisms of thyroid hormone changes during sepsis and consider the place for further study of T3 replacement.