

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

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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 \pm SEM $\mu\text{g}/\text{kg}$; 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.

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23rd March 2015

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.

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“If a job is worth doing, it is worth doing well.”

Lois Maiden (my grandmother)

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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-Triphosphate
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)

$$(\text{MAP} - \text{Right Atrial Pressure}) / \text{CI} [\times 79.9] = \text{dyn.s} / \text{cm}^5 \cdot \text{m}^2$$

CVP was used as an estimate of right atrial pressure

Pulmonary Vascular Resistance Index (PVRI)

$$(\text{mPAP} - \text{Left Atrial Pressure}) / \text{CI} [\times 79.9] = \text{dyn.s} / \text{cm}^5 \cdot \text{m}^2$$

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

Left Ventricular Stroke Work Index (LVSWI)

$$\text{Stroke Volume Index} \times \text{MAP} \times 0.0144 = \text{g.m} / \text{m}^2$$

Right Ventricular Stroke Work Index (RVSWI)

$$\text{Stroke Volume Index} \times \text{mPAP} \times 0.0144 = \text{g.m} / \text{m}^2$$

Respiratory

P:F

$$\text{PaO}_2 (\text{mmHg}) : \text{FiO}_2$$

Minute Ventilation

$$\text{Tidal Volume (V}_T) \times \text{Ventilation Rate} = \text{mL} / \text{minute}$$

Pulmonary Compliance

$$\text{Tidal Volume (V}_T) / \text{Plateau Inspiratory Pressure} = \text{mL} / \text{cmH}_2\text{O}$$

Renal

Creatinine Clearance

$$\frac{[\text{Urine Creatinine concentration (mmol/L)} \times \text{Urine flow rate (mL/min)}]}{\text{Serum creatinine concentration (mmol/L)}} = \text{mL/min}$$

Fractional Excretion Na^+

$$\frac{[\text{Urine } \text{Na}^+ \text{ concentration (mmol/L)} \times \text{Serum Creatinine concentration (mmol/L)}]}{[\text{Plasma } \text{Na}^+ \text{ concentration (mmol/L)} \times \text{Urine Creatinine concentration (mmol/L)}]}$$

Metabolic

O_2 Delivery (DO_2) Index

$$[1.39 \times \text{Hb (g/L)} \times \text{SaO}_2 + (0.003 \times \text{PaO}_2)] \times \text{CI} = \text{mL / min / m}^2$$

O_2 Consumption (VO_2) Index

$$[1.39 \times \text{Hb} \times (\text{SaO}_2 - \text{SvO}_2)] \times \text{CI} = \text{mL / min / m}^2$$

O_2 Extraction Ratio

$$(\text{SaO}_2 - \text{SvO}_2) / \text{SaO}_2$$

Anion Gap

$$[\text{Na}^+ + \text{K}^+] - [\text{Cl}^- + \text{HCO}_3^-]$$

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	x 0.015
		0.8 – 2.0 ng/mL	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	x 0.0154
		0.14 – 0.3 ng/mL	x 1.536
Total T4	58 – 160 nmol/L	4.5 – 12.5 µg/dL	x 12.87
		45 – 125 ng/mL	x 1.287
Free T4	10 – 23 pmol/L	0.8 – 1.8 ng/dL	x 12.87
		80 – 180 ng/mL	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.