Tocotrienols and Neuroprotection

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Stroke: major cause of disability and death

More than 80 % ischemic in nature

Basic strategy in acute treatment is:

- to quickly restore blood flow to the focal area
  - eg through lysing the arterial thrombus
  - alteplase

- to prevent/minimize injury of the brain tissues
  - through using a neuroprotectant
No clinically proven neuroprotectant

- > 1000 compounds shown neuroprotective in cell and animal studies but none succeeded in human trials (O’Collins et al, 2006)

- Compounds tested but failed include:
  - NMDA antagonists
  - calcium antagonists,
  - sodium and potassium channel blockers
  - glycine and serotonin receptor antagonists
  - antioxidants and radical scavengers
  - GABA receptor agonists
  - antiinflammatory agents etc

Shown to significantly reduced infarct volume in animal studies but disappointing in clinical trials.
Reasons for failures

- Brain of animals different from human
  - rodents has ~10% white matter, humans ~50%
  - rodent studies display protection mainly on grey matter
  - agent may not also be protective to white matter
  - white matter is a major component affected in human stroke (disruption of motor and sensory functions)

**NEW PARADIGM:** protect not only grey but also white matter
Other reasons

- Excessive doses used in animal studies
- Unrealistic concentrations in cell studies
Another major reason for failure

- Many evaluated for acute treatment of ischemic stroke
- But stroke has short treatment time window (~ 4.5 hrs)
- Poor blood perfusion during an ischemic stroke may limit the agent from reaching the target
- Thus, acute ischemic stroke not a good study model

Agent should best be given *before* and *not after* a stroke event *(like in most animals studies)*
Prophylaxis may be a better strategy

- Especially for high-risk populations: *those with atrial fib, TIA or undergoing angioplasty or coronary by-pass or even those with CV risk factors*
- Antiplatelet, anticoagulants commonly used
- But for long term neuroprotection: *no pharmacological agent is yet available*
- Vitamin E tocotrienols have shown potential
Quick Background of vitamin E

- First discovered in 1922 (Evans & Bishop)
- Deficiency can lead to:
  - loss of fertility
  - muscular dystrophy
  - encephalomalacia
  - neurological abnormalities: areflexia, ataxia

Vitamin E essential for normal functions and health of our nervous systems
Vitamin E consists of 8 isoforms

4 tocopherols

4 tocotrienols
Tocotrienols (T3) versus tocopherols

A scan of the scientific literature revealed that tocotrienols have some unique biological activities compared to tocopherols.
A notable example

- \( \alpha\text{-tocotrienol} \) but \textbf{not} \( \alpha\text{-tocopherol} \) at nM conc shown to protect neurons from degenerating when challenged with glutamate \( \text{(Sen et al, 2000)} \)

- Mechanism: through \textbf{modulating chemical signals} within the neuronal cells: C-src kinase \( \text{(Sen et al 2000)} \) \text{and} \( 12\)-LOX \( \text{(Khanna et al 2003)} \)

- Later studies in rodents \( \text{(khanna et al 2005)} \) and dogs \( \text{(Rink et al 2011)} \) showed tocotrienols have protective effects against stroke induced neurodegeneration.
Ultimate proof of their genuine worth is still evidence from human studies - forms basis of our study
Our own animal studies: T3s well distributed

\[ \alpha\text{-T3} = 4.5\text{mg} \]
\[ \gamma\text{-T3} = 11.3\text{mg} \]
\[ \delta\text{-T3} = 3.0\text{mg} \]
Biological half-life in humans

Half-life of T3 much shorter than α-tocopherol

- α-tocopherol: > 20 hrs
- α-tocotrienol: 4.4 hrs
- γ-tocotrienol: 4.3 hrs
- δ-tocotrienol: 2.3 hrs

Twice daily dosing is recommended
Neuroprotective study in human subjects

commenced > 3 years ago

collaborative effort:
- Universiti Sains Malaysia
- Malaysian Palm Oil Board (MPOB)
- HOVID Berhad

( Study funded with a grant from MPOB )

( http://clinicaltrials.gov/ct2/show/NCT00753532)
Biggest challenge: suitable human model

- Inappropriate model will lead negative results
- Add tocotrienols to list of failed compounds
- Earlier work remained as publications, soon forgotten
- Suitable model should have the following attributes
  - involve lesions of brain tissues
  - quantifiable non-invasively eg MRI
  - self-progressive, hence no induction
  - permits agent to be evaluated prophylactically

*white matter lesions fit the bill*
What are White Matter Lesions (WMLs)?

- Many older people have hyper-intensities (bright spots) in their brain MRI images, even with no apparent symptoms.
- Lesions formed from bundles of nerve fibers degenerating due to small blood vessel disease of the brain.
- Hence, can be utilized for prophylactic studies of neuroprotectants.
samples of MRI images (top=normal, bottom with WMLs)
Measurable outcome?

Volume of lesions on repeated MRI

If tocotrienols are neuroprotective, should have lesser increase/progression in the volume of lesions compared to the placebo group
Our Study

- Recruited 121 volunteers with WMLs (MRI+ve)
  - Above 40yrs with one or more of: BMI>25, hypertensive, diabetic or hypercholesterolemic
  - Randomised 200mg of mixed tocotrienols (Tocovid Suprabio) twice daily or placebo
  - MRI at baseline, repeated 1 year and 2 years

- Recruit 120 volunteers with no WMLs (MRI-ve) - radiologist
  - Above 40yrs with one or more of: BMI>25, hypertensive, diabetic or hypercholesterolemic
  - Randomised 200mg of mixed tocotrienols (Tocovid Suprabio) twice daily or placebo
  - MRI at baseline and repeated at 1 year

Both studies: double-blind placebo controlled
Imaging performed using our university MRI
Volume of lesions quantified using validated Endeavor software

1. Plug in MRI file
2. Creates 3-D image
3. Identifies WML
4. Compute WML volume
Example of computed WML volume of a volunteer

<table>
<thead>
<tr>
<th>MRI Image</th>
<th>Lesion Volume (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Baseline</td>
<td>854.5</td>
</tr>
<tr>
<td>2 1 year</td>
<td>676.4</td>
</tr>
<tr>
<td>3 2 years</td>
<td>550.6</td>
</tr>
</tbody>
</table>
Our findings

MRI –ve group (113 out of 120 completed study)

52 were on placebo
17% developed WMLs

61 on Tocotrienols (T3)
31% developed WMLs

- more in T3 group developed WMLs but p > 0.05, NS

- however, volume of lesions in placebo group significantly larger!
Average WML volume (mm$^3$) in subjects who developed WML

- Placebo (n=9)
- T3 supplementation (n=19)

$> 4$ fold difference

$\text{P} < 0.05$

Khanna et al (2005)

Rink et al (2011)
MRI positive group

- 121 recruited (T3 = 62, placebo = 59)
- 109 completed 1 year
- 88 completed 2 years
- Data analyzed according to
  - per protocol (n=88)
  - intention to treat principles (n = 121)
Mean volume of lesions (mm$^3$) at baseline, Yr 1, Yr 2

- Placebo (n=42)
- T3 (n=46)

Per protocol

$p < 0.05$
Average change in volume of lesions from baseline ($\text{mm}^3$)

- **Placebo (n=42)**
  - YEAR 1: 172
  - YEAR 2: 381

- **T3 (n=46)**
  - YEAR 1: 10
  - YEAR 2: -29

Significance: $p < 0.05$

**Note:** The graph shows the average change in volume per protocol.
Mean volume of lesions (mm$^3$) at baseline, Yr 1, Yr 2

- Placebo (59)
- T3 (62)

P < 0.05
Average change in volume of lesions from baseline (mm$^3$)

Year 1

- Placebo (n=59): 122
- T3 (n=62): -17

Year 2

- Placebo (n=59): 270
- T3 (n=62): -46

*P<0.05

intention to treat
Summary

The 2 studies provided **objective evidence** that the tocotrienols are neuroprotective in humans, particularly in the WM region.

Previous animal studies already proven the tocotrienols to be protective in the **grey matter**.

**Taken together, tocotrienols** may help to minimize the **extent of tissue injury in the brain during a stroke event** and hence the clinical outcome.

Tocotrienols are not drugs, natural vitamin E isoforms, safe to be taken as a long term neuroprotective supplement.
A point to note

- Like all fat soluble vitamins, oral bioavailability of tocotrienols is poor and erratic.
- Hence, their usefulness can be limited by their absorption.
Absolute oral bioavailability

from animal studies (oral vs IV):

Alpha-T3 : 27.7%  Gamma-T3 : 9.1%  Delta-T3 : 8.5%

Bioavailability can be enhanced with special delivery system
Fig. 1c Mean plasma alpha-tocotrienol versus time curves of the conventional preparation and Formulation X

- **Conventional Preparation**
- **Formulation X**

Approx 3 fold increase

**Tocovid Suprabio vs normal softgel**

Normal softgel

Tocovid Suprabio
Gamma-tocotrienol

Fig. 1b Mean plasma gamma-tocotrienol versus time curves of the conventional preparation and Formulation X
Delta-tocotrienol

Fig. 1a Mean plasma delta-tocotrienol versus time curves of the conventional preparation and Formulation X
Significance of using such a system

- borne out by 2 separate human studies of T3 on arterial compliance (stiffness)
- Both studies placebo controlled involving 36 human volunteers
- no significant effect observed with a normal softgel preparation (*Rasool et al 2006*)
- significant improvement observed with Tocovid Suprabio although doses used were lower (*Rasool et al 2008*)
Our new ongoing study

Effects of tocotrienol supplementation on peripheral neuropathy and cognitive dysfunction in type I and type II diabetic patients

(funded with a grant from PEMANDU)

- Collaboration with CRC of Seberang Jaya Hospital
- Co-principal investigator: Dr Irene Looi (neurologist)
- started recruitment of patients in Dec 2011
- recruited about 200 patients to date
- target = 300 patients
another new study

- Effects of tocotrienols on rehabilitation outcomes in post moderate stroke patients
- Proposal submitted to ethics committee mid May 2013

Hopefully these trials will provide more clinical evidence on the usefulness of tocotrienols as a neuroprotective supplement
MRI of my brain

Now taking 200 – 400mg T3 daily
Other team members

**USM:** Prof Ibrahim Lutfi, Dr Nurzalina, Dr Enrico Magosso, Mr Yogeswaran, Dr Ansari, Dr Liong (ex-UKM)

**MPOC:** Dr Sundram

**Hovid:** Dr Wong Jia Woei, Dr Ng Bee Hong

Acknowledge assistance from my other postgrad students
Thank You for your attention

“ I hear, I forget
I see, I remember
I do and I understand ”

Confucius