Angiogenesis as a therapeutic option for CTO

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I have no conflicts of interest to declare
Chronic Total Occlusions are not the same as routine angioplasty.
Good collaterals are associated with improved symptoms

Can we promote angiogenesis/arteriogenesis to enhance antegrade collaterals and improve symptoms?
Collateral Vessels

Stent

Concept: Pro-angiogenic drug-eluting stent

- AHG concept 2007
- Do not cross the lesion
- Place DES (angiogenic stimulus) proximal to CTO
- Local delivery
- Enhance antegrade collaterals and flow
AHG Group & PHI Compounds

• Preliminary work:
  PHI Di-methyl oxalylglycine (DMOG)
  – Enhanced collateral vessels in a porcine model of CTO
  – Histological evidence only
  – No mechanistic data
  – DMOG not a drug approved for use in man
  – Kelly et al, 2011

• Current study looks in more depth at two new PHI compounds: FG-2216 and FG-4592
  – Approved for clinical trials (renal anaemia)
Overview of Project

Stage 1. Demonstrate that FG-2216/FG-4592 have a pro-angiogenic effect in-vitro

Stage 2. Drug Delivery Options
- Development and production of polymer-coated stents loaded with compound.
  - Characterisation of elution profile.
  - Testing in an in vivo model

Stage 3. First in man studies
- Production of quality controlled coated stents to clinical trial standards.
  - Phase One trials study in patients (CLI)
Study 1: Effects of prolyl hydroxylase inhibitors on accumulation of HIF-1α

1. Both PHIs resulted in increased levels HIF-1α protein.
2. Observed at a lower concentration in cells treated with FG-4592 compared to FG-2216.

- PHI compounds supplied by Prof. Chris Schofield, Oxford University
- HUVEC treated with either FG-4592 (0-50µM) or FG-2216 (0-500µM) for two hours.
- Western blotting performed on cell lysates to detect HIF-1α protein.
**Study 2: Effects of PHI on accumulation of HIF-1α in ex-vivo tissue**

<table>
<thead>
<tr>
<th>Concentration of FG-4592 (µM)</th>
<th>0</th>
<th>0.05</th>
<th>0.5</th>
<th>5</th>
<th>50</th>
<th>100</th>
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</thead>
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Rabbit aorta incubated with FG-4592 for 2 hours shows accumulation of HIF-1α protein above control levels at 5 and 50µM.

Accumulation of HIF-1α is observed in whole aorta treated with FG-4592.
Study 3: Angiogenesis Assay

- Endothelial Tubule Formation Assay

- Matrigel: basement membrane substrate (laminin, collagen IV)

- Image analysis allows independent measurement of:
  - Tubule Number
  - Total Tubule length
  - Branch point number
  - Loop number
Study 3: Endothelial Tubule Assay

HUVEC cells demonstrated a significantly greater degree of tubule formation in the presence of FG-2216.
Study 3: Endothelial Tubule Assay

- A dose dependent effect was seen, up to 500µM.
- Reproducible results (n>10 separate experiments)
- Treated cells able to proliferate.

Dose dependent increase in EC tubule formation with PHI treatment
VEGF mRNA is up-regulated in cells after treatment with FG-2216

- Endothelial cells treated with 500µM FG-2216
- qPCR performed using primers for VEGF and beta-2-microglobulin.
- Also used Qiagen RT2 Profiler PCR Array for Human Angiogenesis
  - Initial results indicate upregulated genes include: VEGFA, ID1, IFNA1, IL-8, LECT-1

n=3 independent experiments
*p<0.05 compared to 0 and 1 hour

VEGF mRNA is up-regulated in cells after treatment with FG-2216
In vitro Summary

Accumulation of HIF-1 protein

Enhanced endothelial tubule formation

Transcription of VEGF mRNA & other GROWTH FACTORS

Upregulation of VEGF mRNA

Prolyl hydroxylase inhibitors

FG-2216    FG-4592

Prolyl hydroxylase

Proteasomal Degradation
Study 5: Stent Coating & Elution

- Fortimedix Kaon cobalt chromium stents
- 8mm x 1.38mm (unexpanded)
- (up to 2.5mm when expanded)

Need to tailor the polymer
Study 5: Programmable Elution Polymers

Programmable elution polymer (PEP) to be composed of two compounds:

- **Polymer A** – Poly(1-vinylpyrrolidone-co-vinyl acetate) (PnVPA)
  - Hydrophilic

- **Polymer B** - Poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (PVB)
  - Hydrophobic

- Starting polymer ratio of PVB:PnPVA of 97:3 (referred to as **PEP97**) was used
- Stent coating and elution measurement completed by Hemoteq, Germany
Elution curve for FG-4592 with different polymer blends

- PEP97
- PEP 100
- PEP 100/10%
- PEP 100/25%
- Grey line: Desired release
Moving forward to pre-clinical studies
Pre-clinical tests: Hind limb Ischaemia Model

- Rabbit IHL & DES Model
- Planned studies:
  - 3 groups:
    - DES + ligation
    - polymer only + ligation
    - Ligation only
- Efficacy (flow, histology)
- Safety (microhaemorrhage, thrombosis)

(Doppler images: Yang et al., 2009)
In Vivo Pilot Study – Model Development

• Day 0: Delivery of stent via femoral artery

• Day 28: Confirmed stent position

- Aorta
- ‘PHI Eluting stent’ (right iliac)
- Ligation of common femoral bifurcation
Summary

• **Aims:**
  - a novel therapy for CTO
  - deliver a pro-angiogenic DES proximal to the occlusion
  - local release of angiogenic compound
  - enhance antegrade collaterals
  - increase blood flow around the CTO

• **Demonstrated** pro-angiogenic potential in vitro for two PHI, FG-2216 and FG-4592 – **NOVEL data**

• **Developed** novel programmed polymer-coated DES, eluting FG-2216 and FG-4592, which achieve our desired elution profile

• **Developed** an in vivo model to test the efficacy and safety of angiogenic DES

• These studies will lead to pre-clinical data to be used, if positive, for designing first-in-man trials
Acknowledgements

- Professor Anthony Gershlick, Project PI
  - Dr Damian Kelly
  - Dr Nikesh Malik
  - Dr Amerjeet Banning
  - Dr Aisling McMahon

- Professor Stuart Egginton, University of Leeds (*In vivo model*)
- Professor Christopher Schofield, Oxford University (*PHI Compounds*)
- Dr Kadem Al-Lamee (*DES consultancy*)

- British Heart Foundation and NIHR
- Colleagues in Cardiovascular Sciences, University of Leicester
Supplemental Slides