



WP 2 Scientific Coordination

Uli Dirnagl & Mhairi Macrae

Aim:

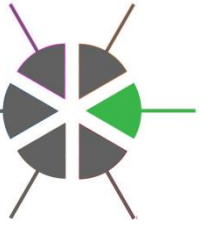
To establish:



the structures and procedures which allow a consortium of trialists to agree on specific investigational drugs or therapeutic principles worth testing by the group



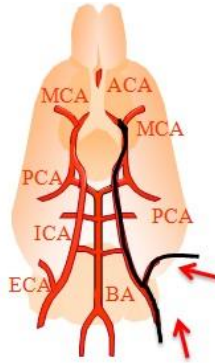
the models, experimental design, and protocols to be used.



Beta - test

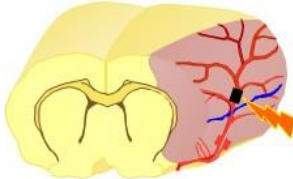
2 mouse stroke models, post-stroke treatment with GTN,
2 time-points (1 & 4hrs post-stroke),
2 functional outcome measures & infarct volume

Intraluminal
filament



Mice: established in 8 centres, limited experience in 1

Direct
electrocoagulation
of distal MCA










Mice: established in 4 centres, limited experience in 1,
3 centres willing to set up

- Standard Operating Procedure templates populated
- Variations in methods between centres identified
- Agreement on options built into SOPs to accommodate variations



Planned Beta test: Protocol for study design

Completed

-  SOPs for mouse tMCAO & pMCAO
-  Inclusion/Exclusion criteria
-  SOP for Humane end-points (currently in draft form)
-  Pre- & post-stroke care package
-  Functional outcome – mouse neuroscore
-  Functional outcome – inverted cage top grip strength
-  TTC stain for infarct volume



Humane end-points

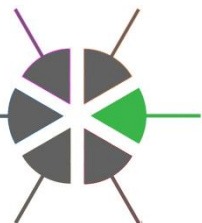
During stroke surgery:

- failure to maintain an adequate depth of anaesthesia
- severe blood loss which significantly compromises normal physiology
- Excessive time taken to complete stroke surgery or atypical behaviour on recovery (e.g. >25 mins for intraluminal filament, >60 mins for distal diathermy)

C grade recorded = Humane end-point

A grade(s) recorded = monitor regularly and PI, vet or care staff alerted

B grade(s) recorded = monitor more frequently, PI & vet must be called in to advise



Berlin Animal Welfare Scale

SYMPTOMS	Grade
1. When checking the animal in the cage	
Animal isolates itself	A
Reduced grooming, secretions around eyes, piloerection	A
Bloated abdomen	A
Slightly increased respiration	A
Urine / faeces differ in quantity, colour and consistency (single or combined)	A
Persistent constipation	A
Food intake uncertain: Weight loss: <10%	A
Hypoactivity	A
Sunken eyes	B
Pallor (eyes, ears, skin)	B
Blood from body orifices, bloody faeces	B
Significantly increased breathing / panting	B
Diarrhoea	B
Unstable and transient apathy	B
Permanent head tilt	B
Paresis in both forelimbs	B
Partial loss of postural reflexes, unable to stand permanently, but animal can reach water and food on their own	B
Paralysis, severe enough to prevent the animal reaching food and water	C
Hypothermia	C

2. During change of bedding/ cages

Small injuries, skin lesions	A
Misalignment of teeth	A
Animal avoids large movements	A
Apathy	B
Stereotypical movement in a circle (circling)	B
Stilted gait	B
Surgical wound complications	B
Local infection (s)	B
Abscess (s)	B
Abdomen hard or tense	B
Dehydration with remaining skin folds	B
Oedema	B
Auto-mutilation	C
Moribund	C
Gasping	C
Loss of postural reflexes	C
Unresponsive to artificial stimuli, touch	C
Pronounced reduction of general condition	C
Ascites (the accumulation of fluid in peritoneal cavity causing abdominal swelling)	C
Paraparesis	C

3. When weighing

Unexplained increase in weight	B
10% weight loss compared to pre-stroke weight or compared to healthy animals of the same strain and age. *	B
Reduced grooming, secretions around the eyes	B
20% weight loss compared to pre-stroke weight or to healthy animals of the same strain and age.	C



SOP for Humane end-points: Points for discussion

1. **Post surgery weight loss** – some groups set humane endpoint at **20%**, others at **30%** but give special diet/hand feeding & consult named vet & animal care staff.

SYMPTOMS	Grade
1. When checking the animal in the cage	
Animal isolates itself	A
Reduced grooming, secretions around eyes, piloerection	A
Bloated abdomen	A
Slightly increased respiration	A
Urine / faeces different consistency (single colour)	A

3 symptoms appear to mean the same thing
Therefore, combine & decide if A, B or C

Comment [IMM5]: Is this the same as Ascites which appears in the next section

Oedema	B
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How is oedema defined ?

2. During change of bedding/ cages	
Ascites (the accumulation of fluid in peritoneal cavity causing abdominal swelling)	C



SOP for Humane end-points: Points for discussion

SYMPTOMS	Grade
1. When checking the animal in the cage	
<u>Hypoactivity</u>	A
2. During change of bedding/ cages	
Apathy	B

Suggest these are combined. A or B ?

<u>Hypothermia</u>	C	Comment [IMM6]: Should we quantify how severe, and time recorded in relation to end of GA?
One suggestion was 30-32° C		
→ Stereotypical movement in a circle (circling)	B	Comment [IMM7]: Should this be included as it is a common sign in stroke animals ?
Stilted gait	B	



SOP for Humane end-points: Points for discussion

Auto-mutilation

C

In some centres this is not a HEP but equivalent to a “B”

Paresis in both forelimbs

B

Paraparesis

C

Paralysis, severe enough to prevent the animal reaching food and water








C

These are probably all equivalent. Suggest combining and set at C



Planned Beta test: Protocol for study design

Still to do

-  SOP for drug administration
-  SOP for randomisation & blinding
-  Plan for Statistical Analysis
-  Set up Steering Committee
-  Set up Data Management Committee (n= 3-4)
-  SOP for removal of blood or tissues at end of experiment
-  Define GO/ NOGO points (Steering Committee to define?)





Proposal for basic post-operative monitoring routine:

On the day of surgery: check the animal every 2 hrs (up to 6 h after surgery) and make notes on a recovery sheet.

1st day post-stroke, monitor and record animal at least 3 times a day

On subsequent days over the following 7 days, monitor and record at least twice a day.