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STROKE DRUG DE-RISKING
& DEVELOPMENT FOR INDUSTRY
CONTACT ME IF YOU REQUIRE ASSISTANCE TO DE-RISK YOUR NEXT ATTEMPT TO DEVELOP A STROKE THERAPY OR IF YOU WANT TO DONATE FUNDS TO HELP TREAT STROKE

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SCREENING IN 2 SPECIES USING MULTIPLE MODELS & MULTIPLE ENDPOINTS

1) RABBIT SMALL CLOT EMBOLIC STROKE MODEL
2) RAT MCAO MODEL

MRI SCANS if required
PK Analysis conducted in 2 species
Toxicity Analysis (Blood chemistry) conducted in 2 species
Safety (hemorrhage-morbidity-mortality) conducted in 2 species
ALL CEDARS-SINAI STUDIES ABIDE BY CURRENT RIGOR GUIDELINES:

Lapchak PA. Recommendations and practices to optimize stroke therapy: Developing effective translational research programs. Stroke. 2013;44:841-843
YOUNG MALE AND/OR FEMALE SUBJECTS
MATURE MALE AND/OR FEMALE SUBJECTS
AGED MALE AND/OR FEMALE SUBJECTS

- BEHAVIORAL ENDPOINTS USING QUANTAL ANALYSIS (HETEROGENEOUS POPULATION ANALYSIS)
- HISTOCHEMICAL ENDPOINTS FOR INFARCT VOLUME AND HEMORRHAGE INCIDENCE (SAFETY OF YOUR DRUG/DEVICE)

RAT MCAO DRUG SCREENING MODEL


RAT MCAO MODEL W/QUANTAL ANALYSIS
YOUNG MALE/FEMALE
AGED MALE/FEMALE

ENDPOINTS: BEHAVIOR USING QUANTAL ANALYSIS AND INFARCT VOLUME USING TTC STAINING
STROKE STATISTICS

- Fourth leading cause of death and leading cause of adult disability in the USA (Roger, Heart Disease and Stroke Statistics—2012 Update. Circulation).
- Annually approximately 795,000 people suffer a stroke (one every 40 seconds with 1 mortality every 3 minutes) in the USA (Lloyd-Jones et al., Circulation 121, e46-e215 (2011)).
- The WHO estimates that 15 million people suffer strokes worldwide annually.
- This results in 5 million deaths and over 5 million patients with chronic disabilities.
EMBOLIC STROKE


(1) **Unknown cause**: 39% of all strokes
(2) **cardioembolic stroke**: 29% of the population
(3) **atheroembolic stroke**: 16% of patients
(4) **lacunar stroke**: 16% of the population

Independent of the type of the ischemic stroke, there is interruption of cerebral blood flow and clinical deficits. An appropriate animal model of embolic stroke should be induced by an embolization or introduction of a blood clot into the brains vasculature.
PathophysiologicaMeasures Using the Rabbit Embolic Stroke Model

- Cerebral Blood Flow
  - Cortical Surface
- Infarcts or Ischemic areas
  - TTC- 2,3,5-triphenyltetrazolium chloride stain
- Brain Energy Metabolism
  - ATP Levels (Mitochondrial Function)
- Behavioral Analysis
  - Quantal Curves
MODELING AN EMBOLIC STROKE

- Common carotid artery ligated, catheter inserted retrograde into common carotid artery- external carotid ligated
- Result- internal carotid artery access to brain
- Blood clots (100-250 µm) are injected through the catheter into cerebral vasculature in unanesthetized rabbits
- Embolization causes numerous infarcts & ischemic cores

Result: Altered pathophysiology, cerebral blood flow and behavioral deficits
Cerebral Blood Flow: Cortical Surface Blood Flow Following Embolization & Reperfusion

Lapchak, Brain Res. 294: 211-7 (2009).
TTC Analysis: Distribution of Infarcts

- Ischemia (Infarct cores) are heterogeneous
  - Standard TTC staining- TTC taken up by mitochondria and converted to RED color- White areas are ischemic tissue.

Lapchak, *Translational stroke research: from target selection to clinical trials*; Lapchak & Zhang, eds. Springer, New York, NY. USA
CORTICAL ATP CONTENT
MEASURED IN ISCHEMIC TISSUE 3 HOURS FOLLOWING EMBOLIZATION

Lapchak PA, De Taboada L. Brain Res. 1306: 100-105 (2010).
Embolic stroke in rabbits:

1) Decreased Cortical Blood Flow
2) Multiple Ischemic cores
3) Loss of ATP in ischemic cores and penumbra

What clinically relevant consequence does embolization have?
BEHAVIORAL OR CLINICAL DEFICITS

- RSCEM uses robust behavioral or clinical endpoint that parallels the NIHSS (motor function component)
- Standard NIHSS is a 15 item impairment scale
- Stroke severity measured by the NIHSS scoring system:
  - 0 = no stroke
  - 1-4 = minor stroke
  - 5-15 = moderate stroke
  - 15-20 = moderate/severe stroke
  - 21-42 = severe stroke
- NIHSS - 16 points rate motor function.

Clinical Rating Scores

- Quantal Analysis Uses a Dichotomous Rating Scale
- Quantal Curves are based upon S-shaped sigmoid logistics curve
- Quantal assay determines how a wide stroke population responds to a treatment
- Neurological Endpoints rated are Motor Functions
  - Ataxia (Lack of coordination of voluntary muscles)
  - Leaning & Circling (Loss of balance)
  - Paraplegia (Total loss of hind limb function)
  - Death is included as an endpoint (<5% incidence)
The figure shows that there is positive correlation between the raw data points circles and the statistically fit sigmoidal quantal curve.
RABBIT EMBOLIC STROKE MODEL

4 THERAPY EXAMPLES

THERAPEUTIC WINDOW CONSIDERATIONS

- tPA: 1-1.5 hour window
- NXY-059: 1 hour window
- Edaravone (Radicut): 3 hour window
- Transcranial laser therapy: 6 hour window

Lapchak PA, Translational Stroke Res 1(2) 96-107 (2010)
**Doppler analysis of MCA blood flow.** The arrow points to the cessation of MCA blood flow when a blood clot occludes or blocks the MCA. This is reversed by tPA administration.

THROMBOLYTIC SIDE EFFECTS

- In the process of thrombolysis, matrix metalloproteinase enzymes (MMP’s) are activated
- Intracerebral hemorrhage (ICH)
- In stroke patients ICH incidence up to 6.5%
- In embolized rabbits ICH incidence varies depending upon the model

Lapchak and Han, Brain Res. 1303, 144-150 (2009).
THROMBOLYTIC EFFECTS IN EMBOLIZED RABBITS: tPA vs. Vehicle

A positive control used in every drug screening study
tPA: thrombolytic (0.9 mg/kg IV; 10% bolus, 90% infused 60 minutes).

Therapeutic Window for AIS: 3 HOURS

As evaluated by the global test statistic, the odds ratio for a favorable outcome in the t-PA group was 1.7 (95 percent confidence interval, 1.2 to 2.6; \( P = 0.008 \)). As compared with the placebo group, there was a 12 percent absolute (32 percent relative) increase in the number of patients with minimal or no disability in the tPA group on the NIHSS and mRS.

**NEJM 333:1581-1588, 1995**
- tPA: thrombolytic
- Therapeutic Window for AIS: 3-4.5 HOURS

The median time for the administration of tPA was 3 hours 59 minutes. More patients had a favorable outcome with tPA than with placebo (52.4% vs. 45.2%; odds ratio, 1.34; 95% confidence interval [CI], 1.02 to 1.76; \( P=0.04 \)). In the global analysis, the outcome was also improved with tPA as compared with placebo (odds ratio, 1.28; 95% CI, 1.00 to 1.65; \( P<0.05 \)).

*NEJM 359:1317-1329, 2008.*
tPA is effective in AIS patients with:

(1) cardioembolic stroke: 38% of subjects improved with tPA compared to 28% with placebo

(2) atheroembolic (large vessel occlusion): 40% of subjects improved with tPA compared with 22% with placebo

(3) small vessel lacunar stroke: 63% of subjects improved with tPA compared with 40% with placebo

Saver et al. Stroke 2010, 66.7% of stroke patients treated with tPA within 3 hours show significant improvement compared to 50% in controls (P=0.003).
CONCLUSION

• The RSCEM was originally used to develop tPA and remains an effective model to predict drug efficacy prior to initiating expensive clinical trials.
• Based upon correlative analysis, it is hypothesized that the RSCEM can be used as an effective translational tool for the development of stroke treatments.
• There is an apparent therapeutic window correlation of 2.43 - 3 hours between the RSCEM and AIS patients.
• The RSCEM should be used as a “GOLD” standard translational guide in order to gauge the clinical potential of new treatments.
Rat quantal bioassay

Increase in ischemia duration correlates with improved behavior when drug X is administered post-stroke.
Drug X improved behavior and decreased TTC infarct volume.
CONCLUSION

• Confirmation of drug activity in a second model in a second species

• Direct measurement of behavior when a therapy is administered post stroke

• Direct measurement of infarct volume

• Confirmation external to your own facility for target and efficacy validation
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