

Conflicts of Interest

- None to declare



The
B.R.A.I.N.S.
Research
Laboratory



McGovern
Medical School

Amelioration of Ischemic Stroke Damage Through Inhibition of Interleukin-6 Signaling with Tocilizumab Requires Sex Specific Dosing

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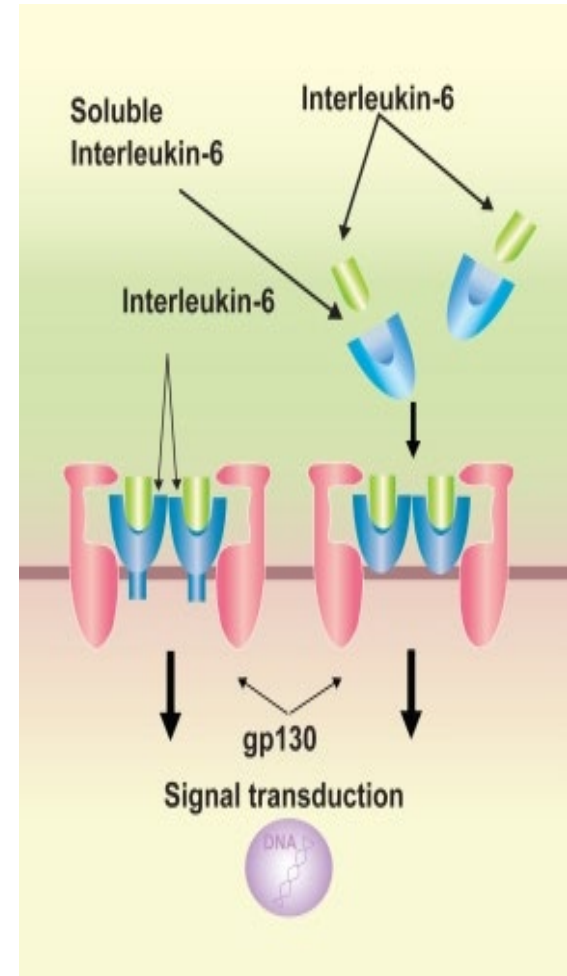
Interleukin-6 and stroke

- Interleukin-6 (IL-6) has been extensively studied in inflammatory disorders both pre-clinically and clinically
- Levels of IL-6 are increased in the plasma of stroke patients when compared to age-matched healthy controls
- Between stroke patients, higher plasma IL-6 levels are correlated with larger infarct volume, increased stroke severity and poor long term prognosis

***IL-6 signaling can be mediated by membrane
and soluble forms of the receptor***

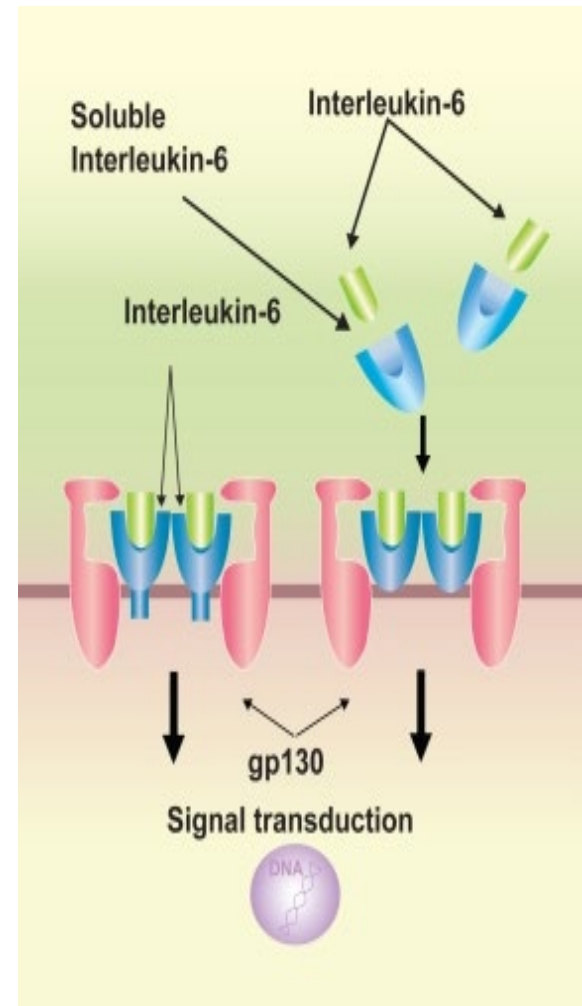
Classical Signaling

- IL-6 Signaling where IL-6 binds directly to the cell membrane (no intermediate)
- Limited to cells which have **IL-6R bound to the gp-130 complex expressed on the membrane** which includes:
 - Neutrophils, monocytes, some T-cells, microglia, astrocytes, neurons and hepatocytes



Trans-signaling

- Signaling of IL-6 using soluble IL-6R
- sIL-6R formed by ***proteolytic cleavage*** of membrane bound IL-6R (***appx. 90%***) and alternative splicing (***appx. 10%***)

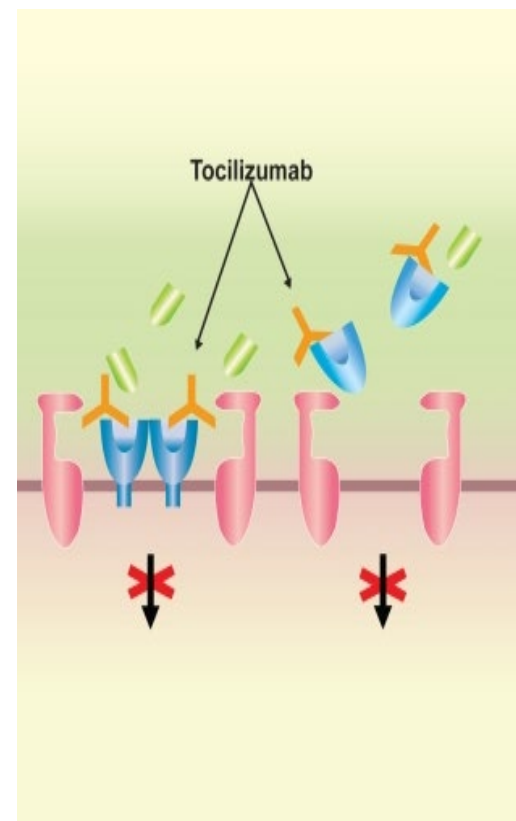


Tocilizumab (Actemra)

IgG1

- Antibody against the Interleukin-6 receptor (IL-6R)
- FDA approved treatment for:
 - *Rheumatoid Arthritis* (onset 40-60 years old)
 - *Giant Cell Arteritis* (onset 70-80 years old)
 - *Systemic Juvenile Idiopathic Arthritis* (onset 1-6 years old)

****** Women are more likely than men to develop both giant cell arteritis and rheumatoid arthritis******

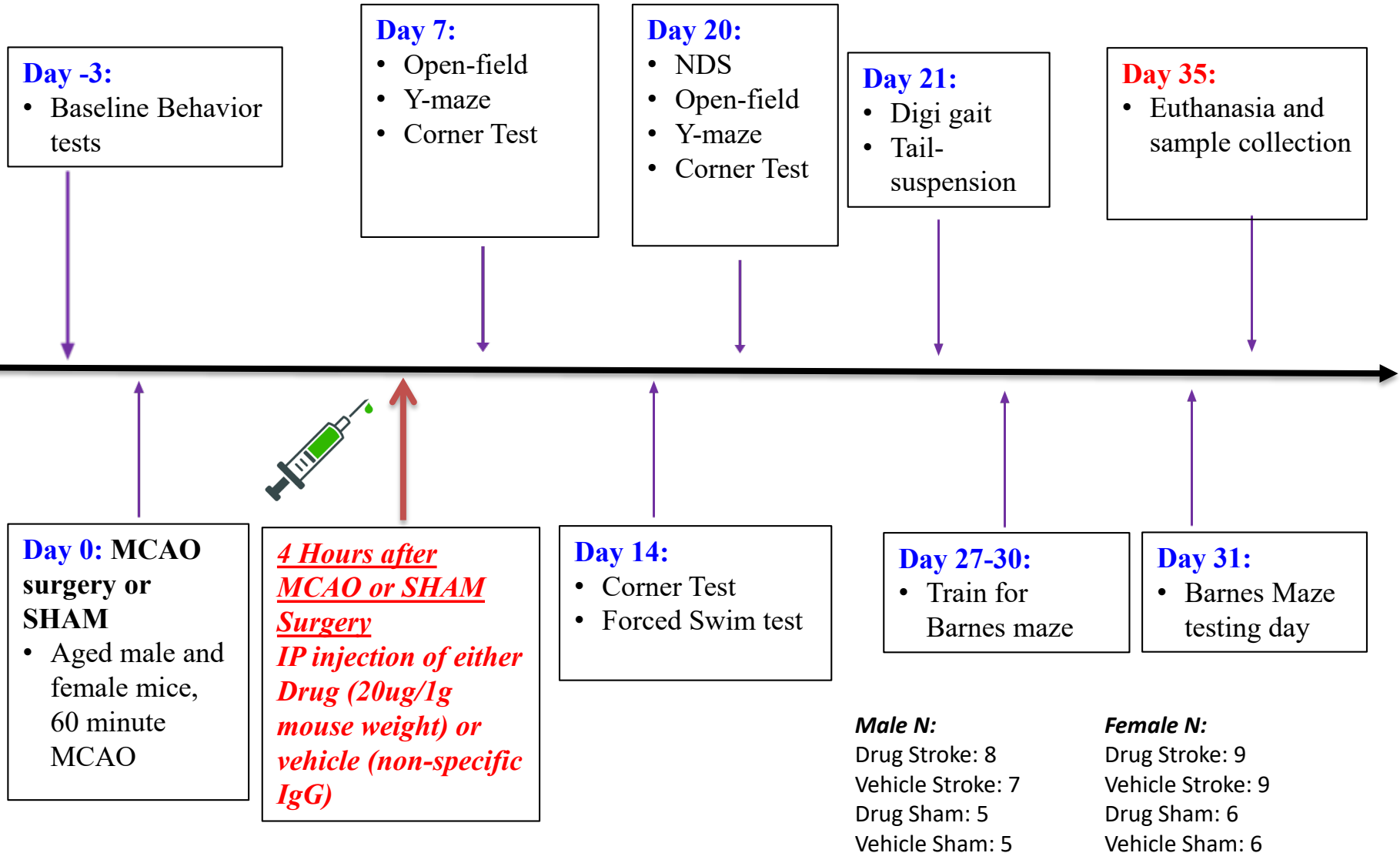


Hypothesis:

Tocilizumab treatment will reduce stroke damage by blocking post-stroke interleukin-6 signaling.

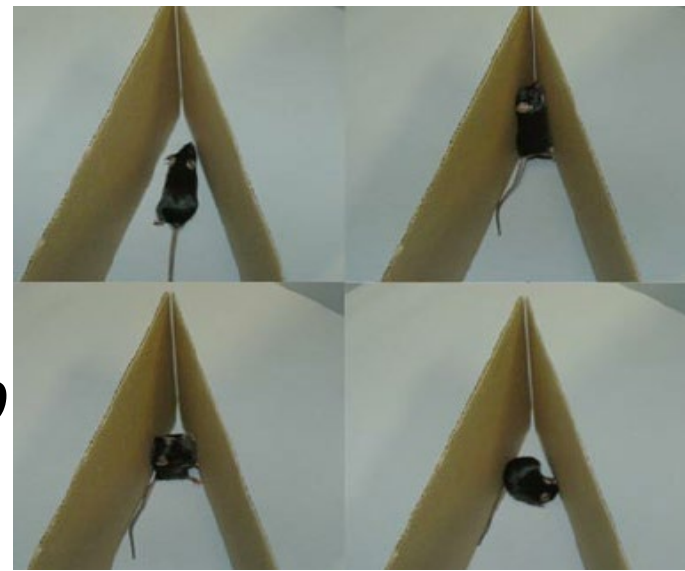
35-day post-stroke survival study

**Using 18-month old males and females*

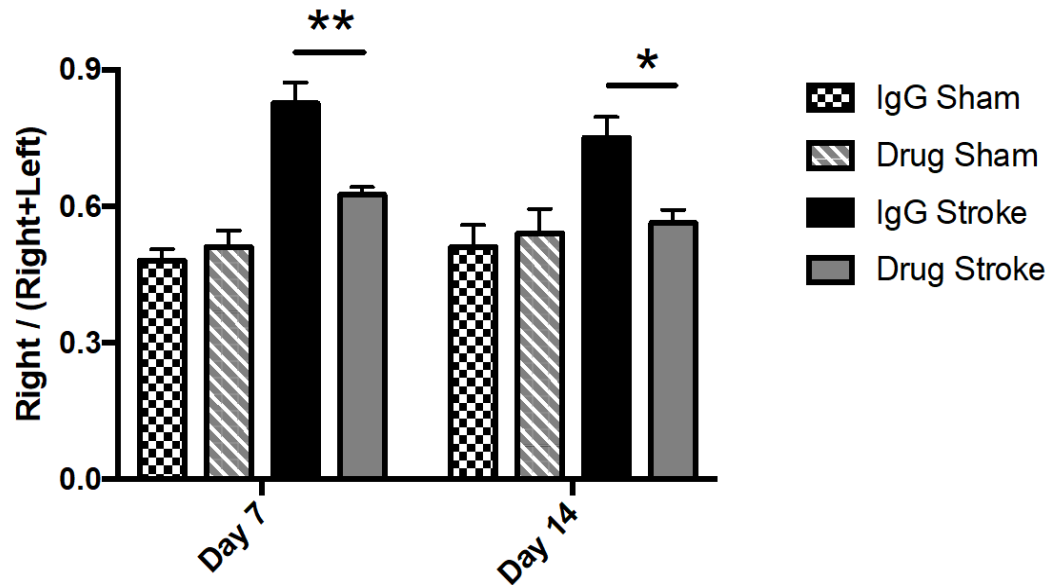


Behavioral changes

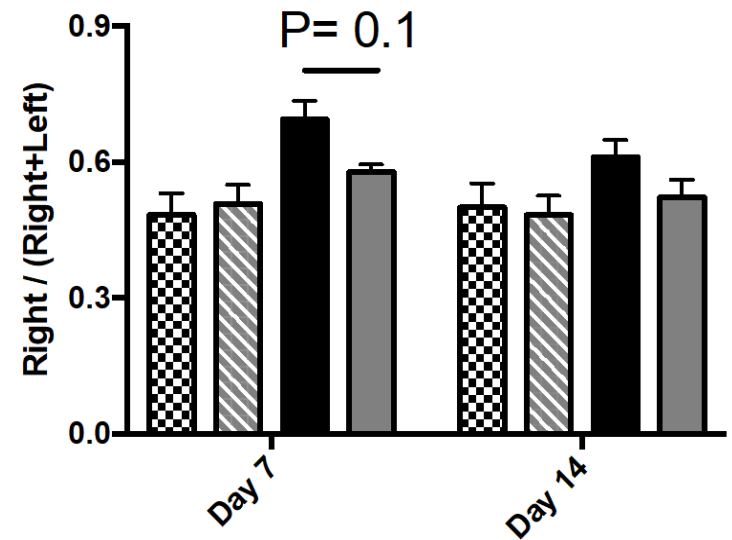
Reduced corner test deficit in aged male mice with tocilizumab



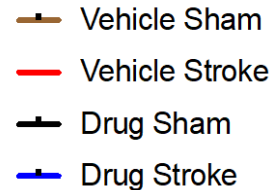
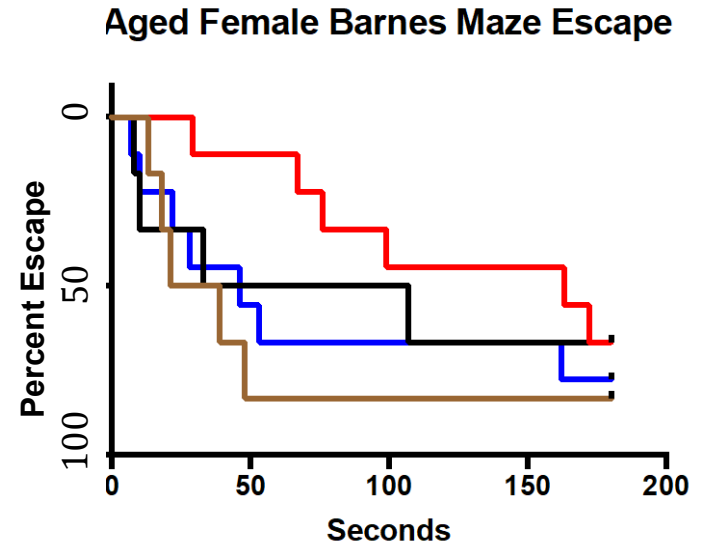
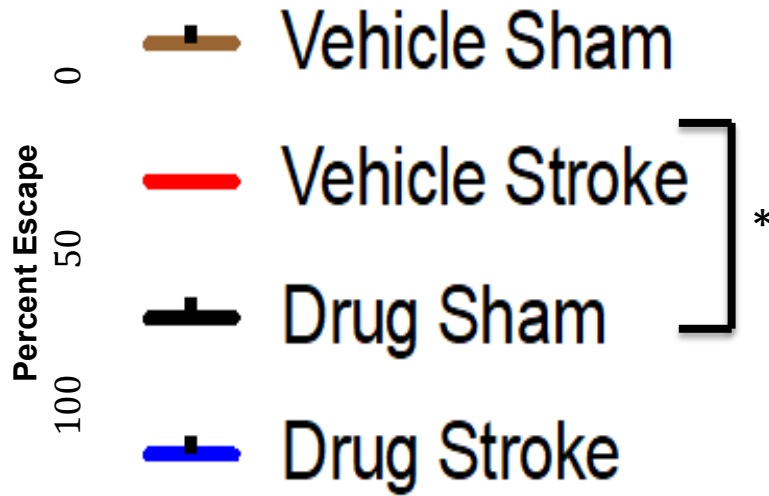
Aged Male Corner Test



Aged Female Corner Test

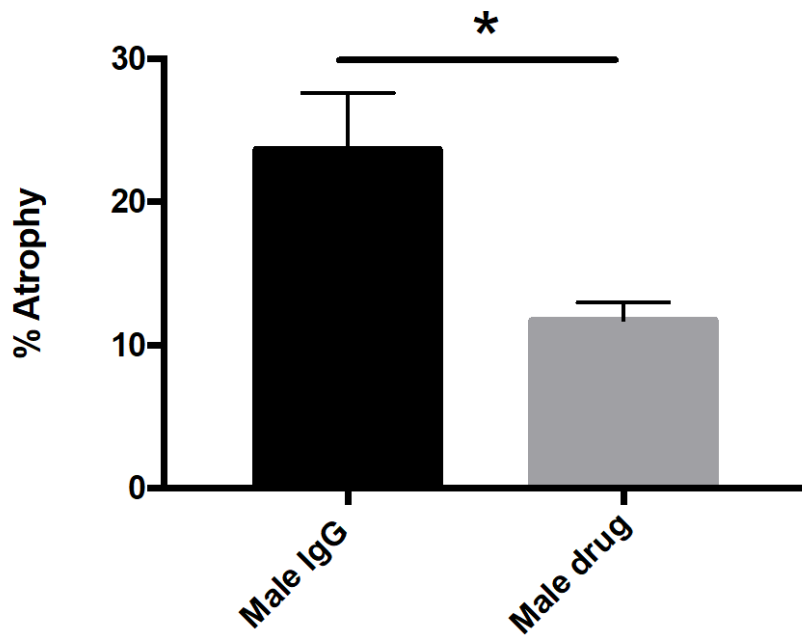


Improved long term cognitive outcomes on the Barnes maze 31-days post-stroke for tocilizumab treated Aged male mice

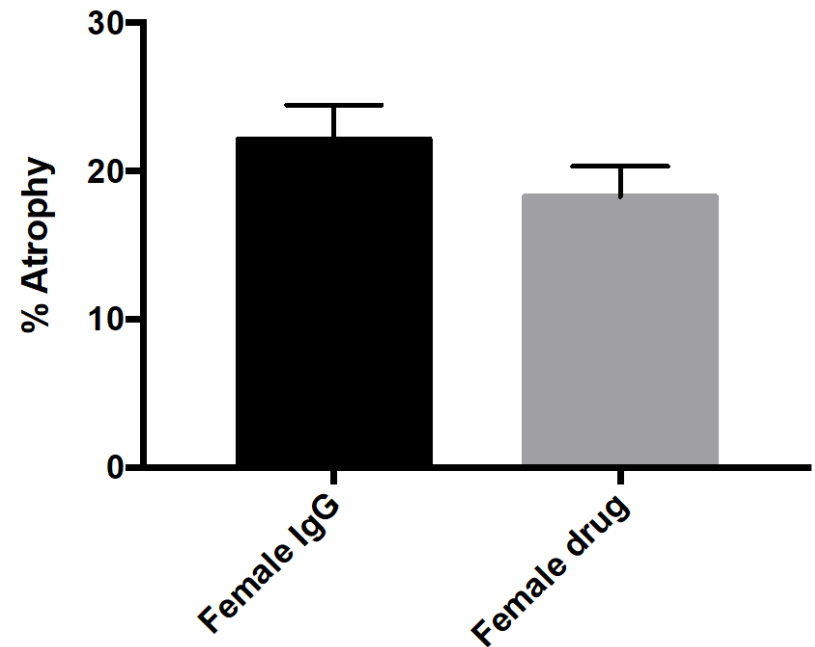


Tocilizumab treatment reduced brain atrophy 35 days post-stroke in aged male mice

35-day Post stroke brain atrophy



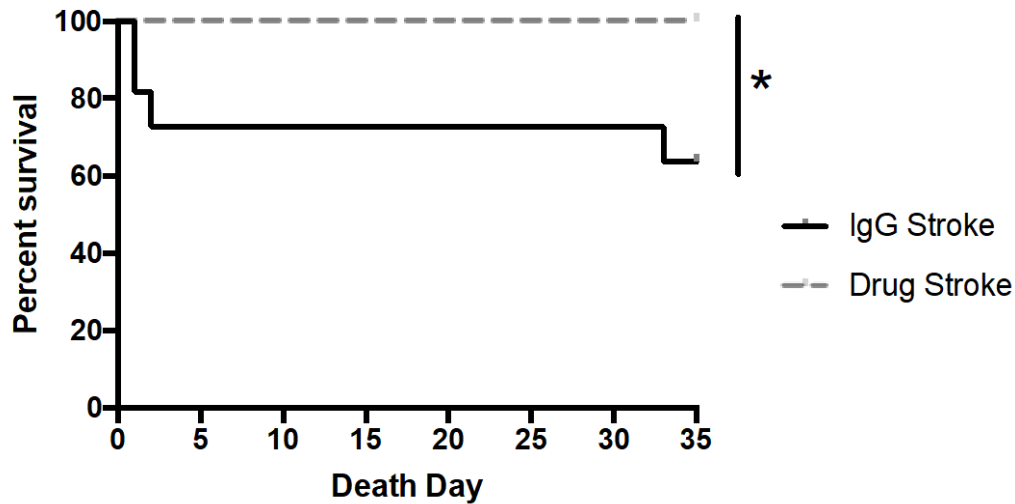
35-day Post stroke brain atrophy



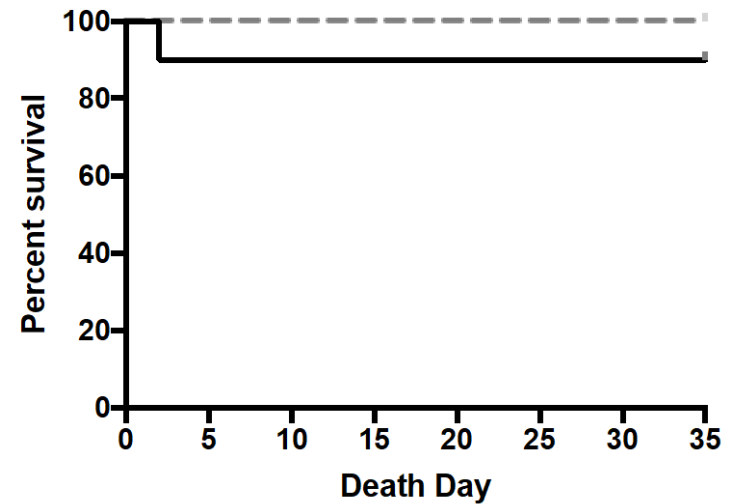
N:
Female Vehicle Stroke: 9
Female Drug Stroke: 9
Male Vehicle Stroke: 7
Male Drug Stroke: 8

Tocilizumab treatment reduced Post-Stroke Mortality in Aged mice

35 day Aged Male Stroke Survival Curve

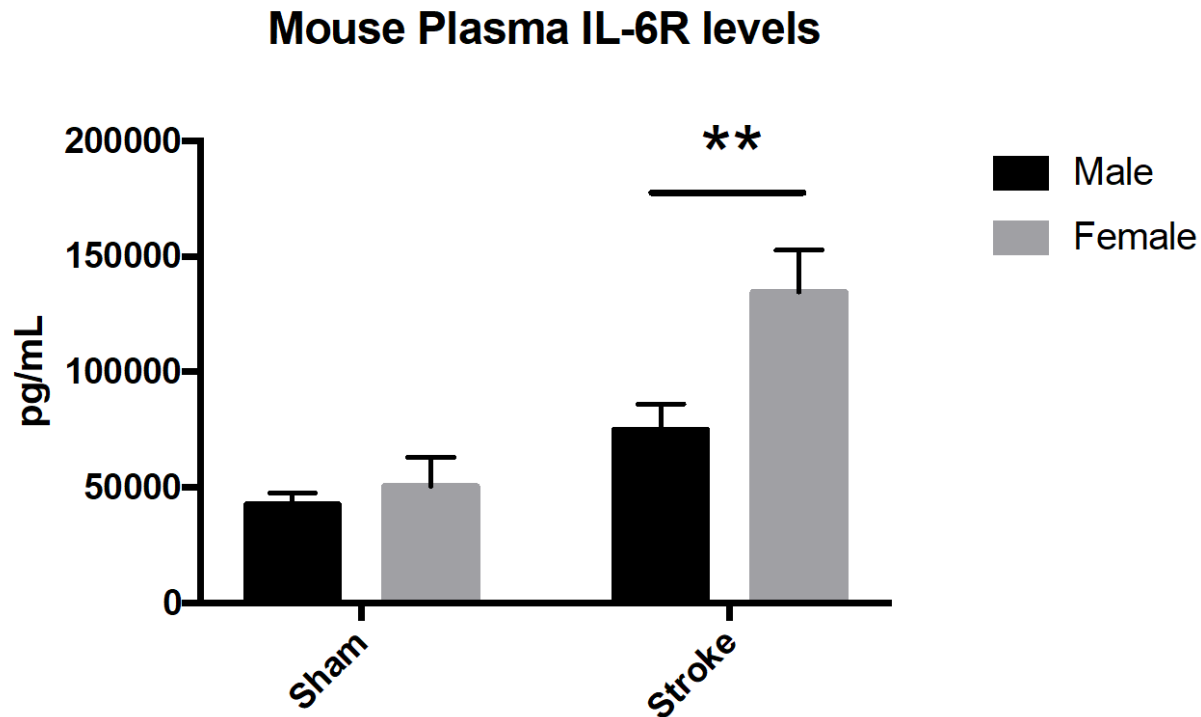


35 day Aged Female Stroke Survival Curve



Why is tocilizumab effective in aged males but not females?

Females have a significantly higher level of soluble IL-6R post-stroke compared to males

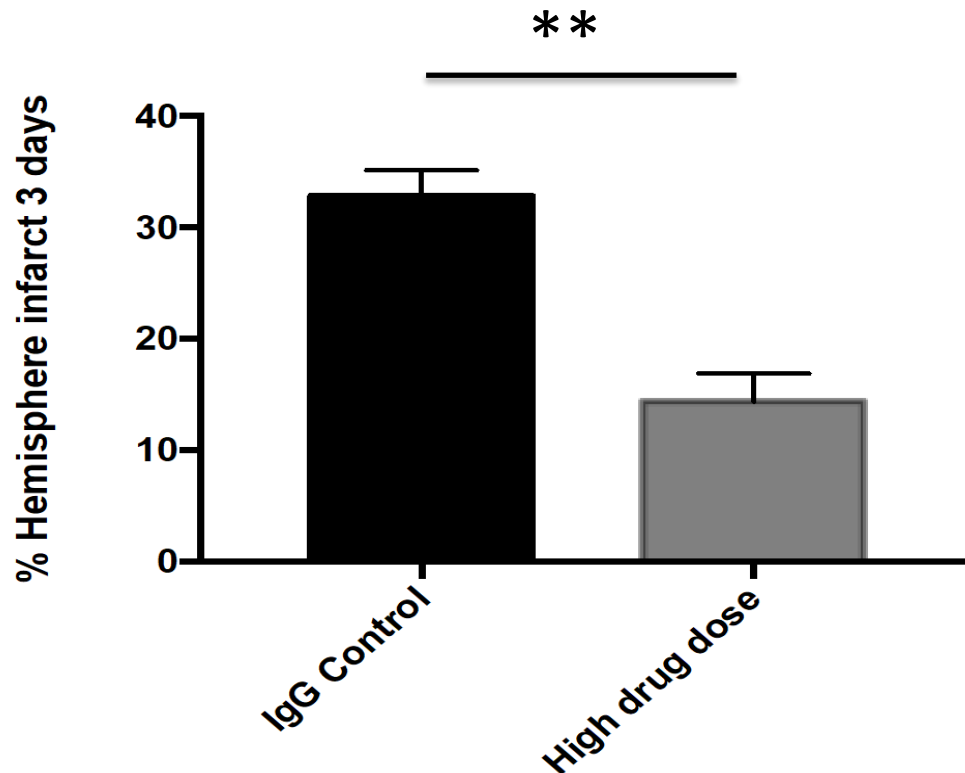


Hypothesis:

- Tocilizumab has been ineffective for aged females so far in the study do to an increase in sIL-6R post-stroke
- A higher dose of tocilizumab (100mg/kg) will lead to reduced infarct in aged female mice 72-hours post-stroke

A higher dose of tocilizumab (100mg/kg) led to significant reduction in infarct 72-hours post-stroke in aged females

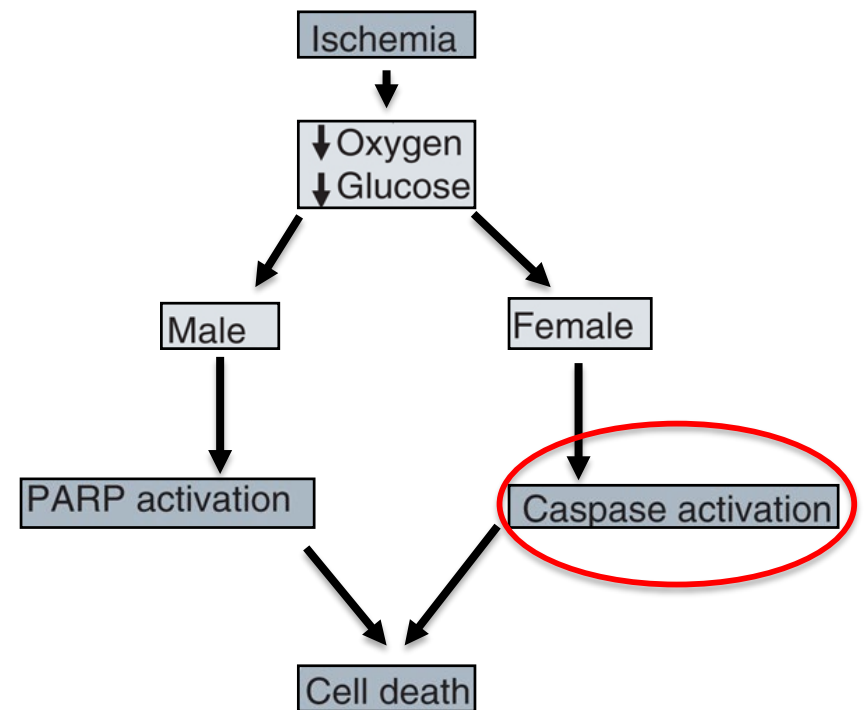
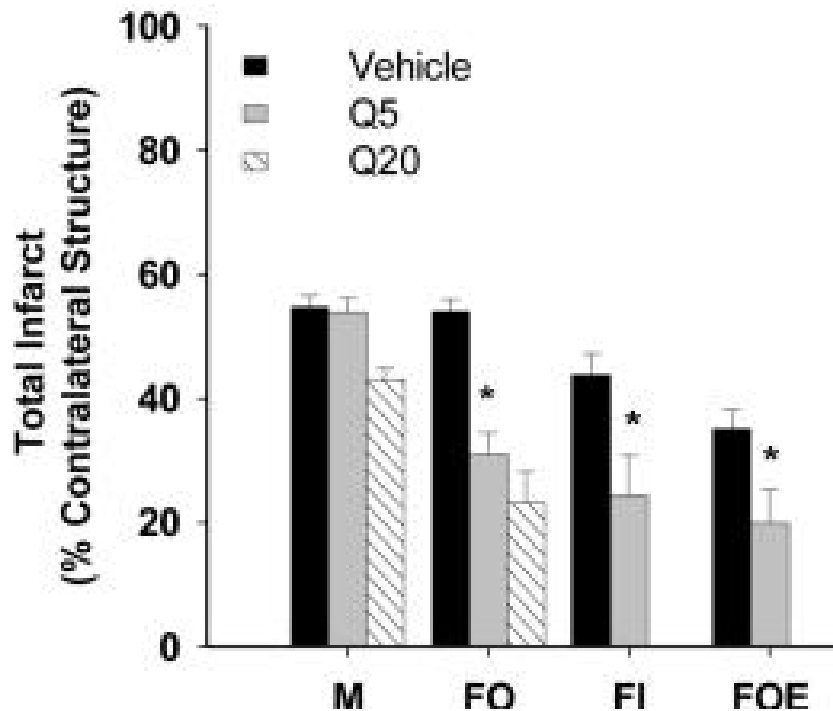
High Drug Dose Female Stroke Test



N:
Drug: 6
IgG control: 5

***Why do females have a higher sIL-6R level
post-stroke compared to males?***

Previous research has shown that cell death pathways differ between males and females, with females undergoing primarily caspase dependent pathways

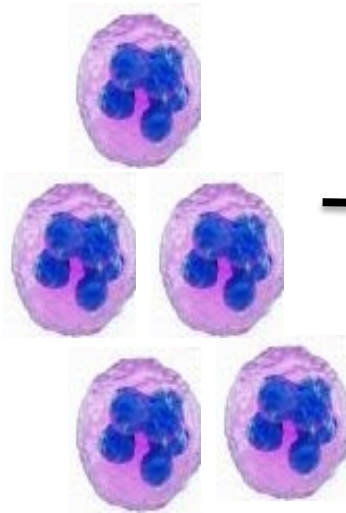
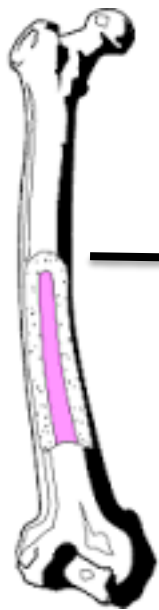


Liu F, Li Z, Li J, Siegel C, Yuan R, McCullough LD. Sex differences in caspase activation after stroke. Stroke. 2009;40(5):1842-8.

Hypothesis:

- Caspase inhibition will lead to a reduction in sIL-6R release in females.
- Since neutrophils release much of the sIL-6R post injury, inhibiting caspase in an ex vivo bone marrow neutrophil assay will reduce the amount of sIL-6R released

Aged male and female ex vivo bone marrow assay

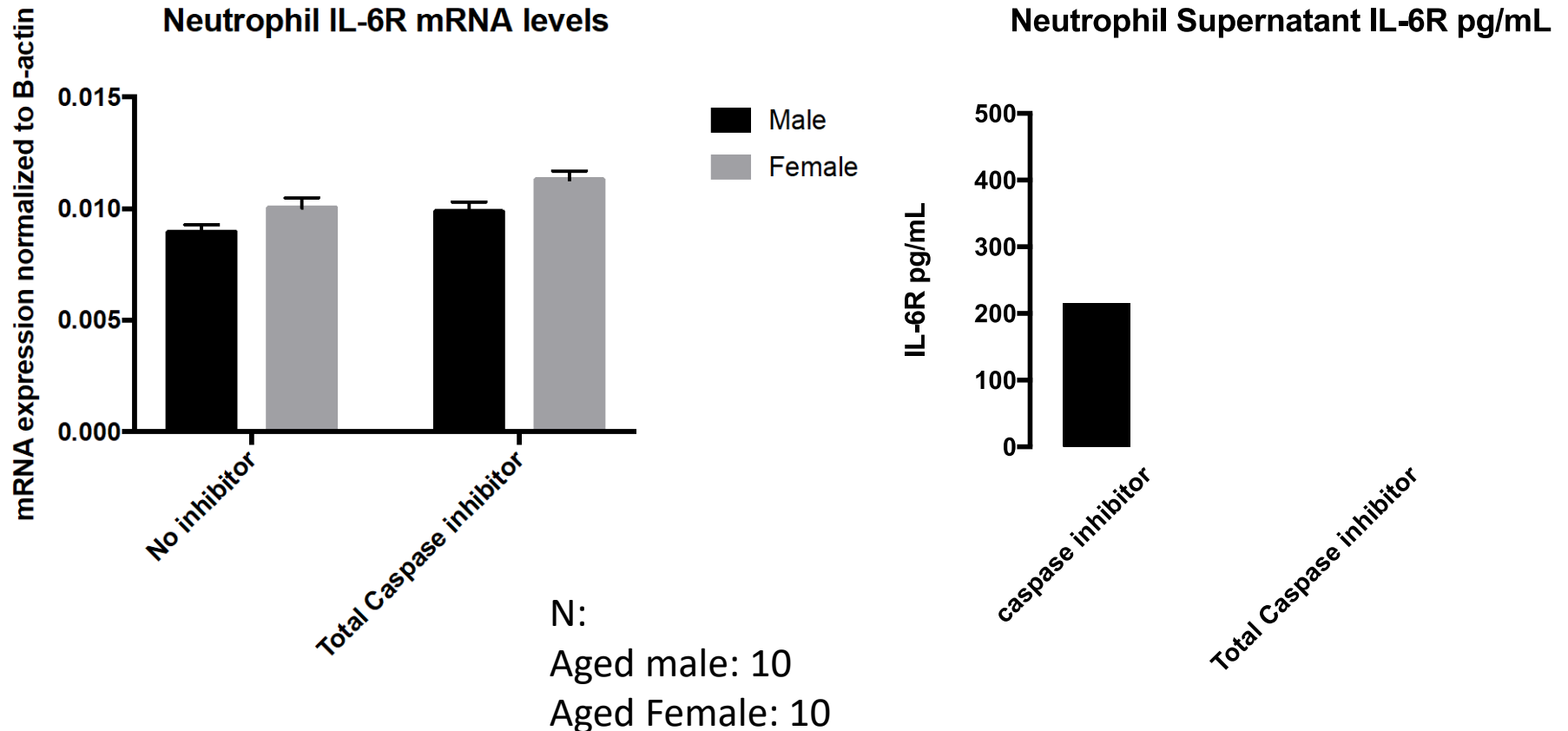


***30 minute
incubation:***
total caspase
inhibitor
(100uM)

1-hour stimulation:
N-Formyl-Met-Leu-
Phe (FMLP) (1uM)

Neutrophils were then collected for mRNA and supernatant for ELISA analysis

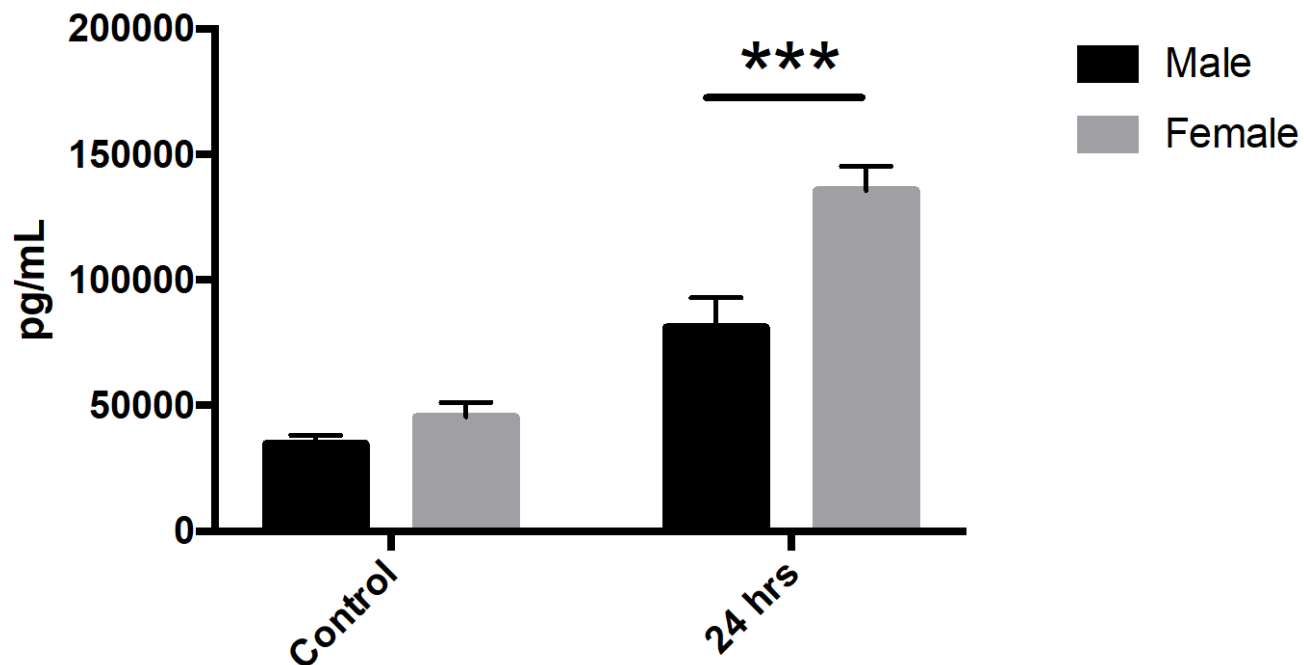
Interleukin-6 receptor shedding, not expression, is caspase dependent in aged female neutrophils but not aged male neutrophils



Clinical Relevance

Women also have a significantly higher level of soluble IL-6R post-stroke compared to men

Human IL-6R Plasma Levels after Ischemic Stroke



Conclusions Drawn from Study

- Inhibiting interleukin-6 signaling with tocilizumab ameliorates injury post-stroke in aged male mice and aged female mice at a higher dose
- Sex difference seen in tocilizumab effectiveness likely caused by higher sIL-6R in females post-stroke a trend also seen in human stroke patient plasma
- If translated into the clinic women would likely need a higher dose than men for therapeutic effect

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