

ABSTRACT

Background: Male sex is an important risk factor in human stroke, yet there are few data on the role of androgens in animal stroke models. We investigated whether testosterone (T) alters infarct volume at 24 hours or 9 days after middle cerebral artery occlusion (MCAO) in male mice treated with surgical castration and androgen replacement. We also determined if androgen effects were mediated through androgen receptor (AR) using AR antagonist, flutamide (F), and the potent, non-aromatizable AR agonist, 5 α -dihydrotestosterone (D). Lastly, we evaluated a novel strain of AR overexpressing mice (AR-TG) to determine if increasing AR in brain would provide neuroprotection as compared to wild type littermates (AR-WT).

Methods: Male C57BL/6 mice received surgical castration (CAST) with androgen replacement via a subcutaneous implant method (dose in mg) 7 days prior to MCAO. In protocol 1, sham castrated (Intact), CAST, CAST+T(1.5), CAST+T(5), CAST+T(1.5)+F(1.5), and CAST+T(5)+F(10), CAST+D(0.5), and CAST+D(1.5) groups were studied, using 90 mins MCAO followed by 24 hours reperfusion. In protocol 2, CAST and CAST+T(1.5) groups received 60 mins MCAO followed by 9 days reperfusion. In protocol 3, intact AR-TG and AR-WT male mice were studied in the same manner as protocol 1. Tissue outcome was analyzed by standard TTC histology with image analysis and quantification of infarct volume as a percentage of contralateral structure. Data was analyzed by one-way ANOVA with post hoc Newman-Keuls test and unpaired t-test for protocol 3. Data are shown as means \pm SEM. The criterion for statistical significance is $P < 0.05$.

Results: Both T (1.5) and T(5) delivered serum total testosterone levels over the physiological range. In protocol 1, infarct in CAST group (Cortex: 43.7 \pm 2.6%, Striatum: 93.4 \pm 3.1%, and Hemisphere: 33.0 \pm 1.9%) was smaller than that of INTACT (55.7 \pm 3.1%, 108.2 \pm 4.6% and 42.2 \pm 2.5%). Cortical and hemispheric infarcts in CAST+T(1.5) were smaller relative to CAST, while CAST+T(5) infarct volume was larger as compared to CAST. Flutamide blocked all tissue effects of testosterone. D also had dose dependent effects as CAST+D(0.5) and CAST+D(1.5) produced similar effects on infarction as in CAST+T(1.5) and CAST+T(5), respectively. In protocol 2, CAST+T(1.5) sustained smaller infarct volumes as compared to CAST. In protocol 3, AR-TG mice demonstrated smaller infarct volumes as compared to AR-WT mice.

Conclusions: Low physiological levels of androgens, T and D, are neuroprotective in male mice, while higher doses increase damage. Both effects are likely mediated through the AR. Supported by NIH grants NS 33668, NR03521, NS49210.

BACKGROUND

Male Androgens in Stroke

- Testosterone (T) decreases after 50 years of age (Morley et al., PNAS1997)
- T levels decrease after acute stroke (Dash et al., Funct Neurol 1991)
- Decreased T levels have also associated with infarct size and poor outcome after acute stroke in elderly men (Jeppesen et al., Arterioscler Thromb Vasc Biol 1996)
- T increases MCA occlusion lesion size in male rats (Cheng et al., JCBFM 2007)
- T accelerates functional recovery after stroke in male rats (Pan et al., Brain Res 2005)

Androgen Receptor (AR) in Brain

- AR distributed widely in the CNS (Finley et al., J Neurobiol 1999)
- AR upregulated after cerebral ischemia-reperfusion injury (Yang et al., J Neurobiol 2005)
- AR downregulated after castration, upregulated linearly dependent on testosterone levels (Lu et al., Endocrinology 1998)

Role of Androgens & AR on the Stroke Outcome remain Unclear

HYPOTHESES

- Physiological levels of T during cerebral ischemia improve both short- and long-term male stroke outcome
- Effects are mediated via AR

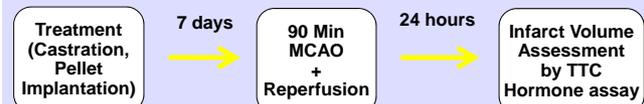
METHODS & RESULTS

- All experiments were performed in accordance with NIH guidelines for research animal care and approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University.
- 22-30 g male C57BL/6 mice approximately 9-10 weeks of age
- 90 or 60 min middle cerebral artery occlusion (MCAO) was performed due to each study protocol under isoflurane anesthesia with laser-Doppler flowmetry
- Head temperature (35.5 – 37.5°C) under left temporal muscle during MCAO and at reperfusion
- Infarct volume was determined by 1.2% 2,3,5-tetrazolium chloride (TTC) staining (% of contralateral structure) at 24 h reperfusion

Short-Term Outcome Study

Groups	Castration	Hormone pellet implantation
INTACT (n=18)	-	-
CAST (n=20)	+	-
T(1.5) (n=20)	+	1.5 mg testosterone
T(1.5)+F(1.5) (n=11)	+	1.5 mg testosterone + 1.5 mg flutamide
T(5) (n=12)	+	5 mg testosterone
T(5)+F(10) (n=12)	+	5 mg testosterone + 10 mg flutamide
DHT(0.5) (n=11)	+	0.5 mg 5 alpha-dihydrotestosterone
DHT(1.5) (n=12)	+	1.5 mg 5 alpha-dihydrotestosterone

Flutamide: AR antagonist
DHT: The potent, non-aromatizable AR agonist



Hormone assay: Total and free testosterone were measured

RESULTS (1)

Free T levels in INTACT, CAST, T(1.5), T(5), DHT(0.5), DHT(1.5) were 0.5 \pm 0.4, 0.0 \pm 0.0, 27.3 \pm 1.2, (medium physiological level) 43.4 \pm 2.6 (maximum physiological level), 0.0 \pm 0.0, and 0.0 \pm 0.0 pg/mL respectively.

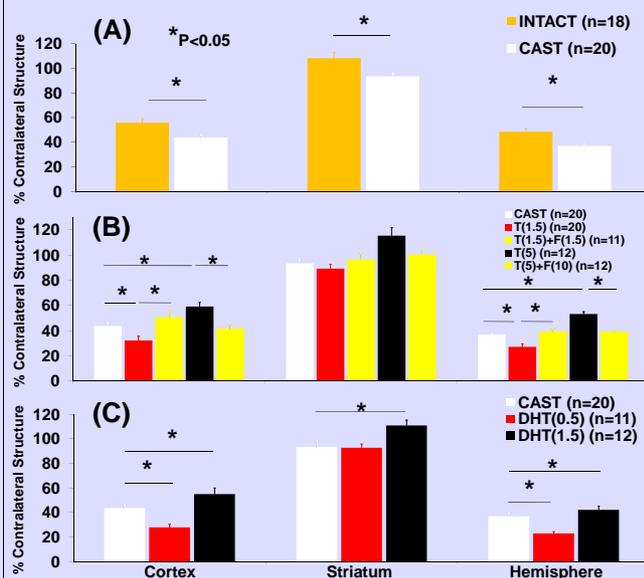
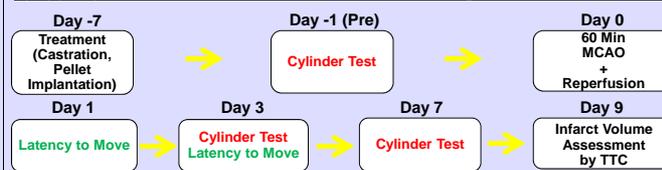


Figure 1. Infarct volumes were assessed at 24 h reperfusion after 90 min MCAO. (A) Castrates sustained smaller infarcts than intact. (B) Dose dependent effects of testosterone were attenuated by androgen receptor antagonist flutamide. (C) Dose dependent effects of DHT.

Long-Term Outcome Study

Groups	Castration	Hormone pellet implantation
CAST (n=18)	+	-
T(1.5) (n=20)	+	1.5 mg testosterone



- 9cm in diameter and 15cm in height cylinder
- Four cameras placed around the cylinder
- Maximum of two paw touches for one rearing were recorded
- A total of 20 touches was recorded during 10 min
- Left / total paw touches was calculated



RESULTS (2)

Sustained smaller infarct volume was seen in T(1.5) (20.0 \pm 1.4%) than CAST (29.6 \pm 1.2%) in the total hemisphere at 9 days reperfusion after 60 min MCAO.

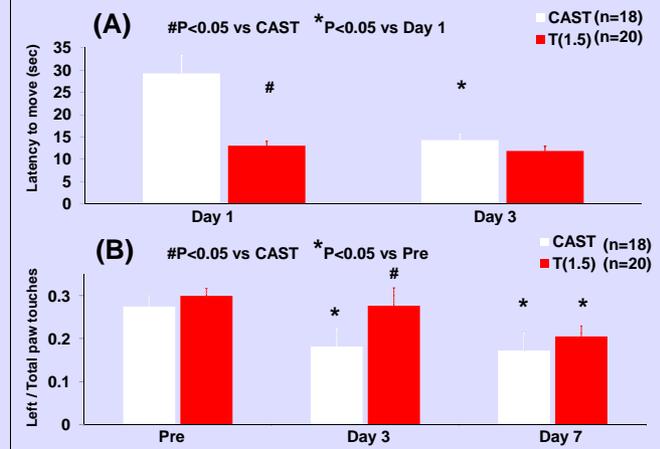


Figure 2. Motoneuronal function was assessed before and/or after 60 min MCAO in (A) latency to move and (B) cylinder test. (A) CAST showed impairment on Day 1 and recovery by Day 3, while T(1.5) showed no impairment on either time point. (B) CAST showed impairment by Day 3, while T(1.5) showed no impairment on Day 3 but impaired by Day 7.

AR-TG (AR-overexpressing) Mice Study

Intact male 22-30 g, 9-15 weeks of age, AR-WT (n=10) and AR-TG (n=10) were studied in the same way as the Short-Term Outcome Study.

AR-TG mice were generated from matings of C57BL/6 males and DBA/2J females. Rat AR cDNA was cloned downstream of a 3.6-kb alpha1(I)-collagen promoter fragment and used to create AR-TG mice. (Wiren et al., Endocrinology 2004)

RESULTS (3)

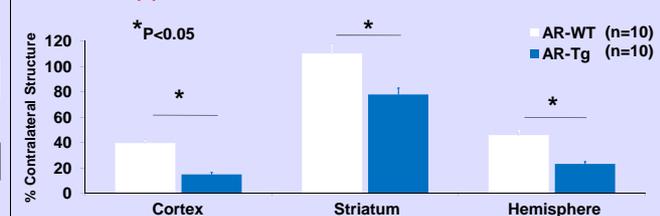


Figure 3. Infarct volumes were assessed at 24 h reperfusion after 90 min MCAO. AR-TG males showed smaller infarcts than AR-WT males.

CONCLUSIONS

- Low dose androgen reduces infarct size in male brain
- Signaling pathway occurs through the AR
- Effect of low dose testosterone on functional recovery may be beneficial to motor function in early time point

This work was supported by NIH grants NS 33668, NR 03521, and NS 49210.