# Sex-Specific Differences to Ischemic Stroke

Subjects: Cell Biology

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Macroautophagy (called autophagy thereafter) is a self-catabolic process where subcellular proteins, macromolecules, and organelles are sequestered within membrane-enclosed vesicles (autophagosomes) and are degraded by fusion with lysosomes (autolysosomes). Autophagy plays a role in cellular homeostasis by degrading damaged cellular contents and redistributing the constituents for other cellular processes. During times of cell stress, such as ischemia, autophagy may become dysregulated and increase injury, or conversely may increase the ability of the cell to survive under conditions with low energy substrates. There is increasing evidence that autophagy is a sex-dependent process.

Keywords: autophagy ; middle cerebral artery occlusion ; 3-methyladenine ; sex differences ; ischemic stroke ; neuroprotection

## 1. Overview

Ischemic stroke triggers a series of complex pathophysiological processes including autophagy. Differential activation of autophagy occurs in neurons derived from males versus females after stressors such as nutrient deprivation. Whether autophagy displays sexual dimorphism after ischemic stroke is unknown. We used a cerebral ischemia mouse model (middle cerebral artery occlusion, MCAO) to evaluate the effects of inhibiting autophagy in ischemic brain pathology. We observed that inhibiting autophagy reduced infarct volume in males and ovariectomized females. However, autophagy inhibition enhanced infarct size in females and in ovariectomized females supplemented with estrogen compared to control mice. We also observed that males had increased levels of Beclin1 and LC3 and decreased levels of pULK1 and p62 at 24 h, while females had decreased levels of Beclin1 and increased levels of ATG7. Furthermore, the levels of autophagy markers were increased under basal conditions and after oxygen and glucose deprivation in male neurons compared with female neurons in vitro. E2 supplementation significantly inhibited autophagy only in male neurons, and was beneficial for cell survival only in female neurons. This study shows that autophagy in the ischemic brain differs between the sexes, and that autophagy regulators have different effects in a sex-dependent manner in neurons.

## 2. Macroautophagy

Epidemiologic and clinical evidence have demonstrated the importance of sex differences in the incidence and response to ischemic brain injury [1][2][3]. Women have lower stroke incidence relative to men until well after menopause; however, rates climb dramatically in elderly women who also have greater disability, morbidity and mortality after stroke than men [2][4]. Previous studies have suggested that sex-dependent pathways are activated in response to stroke, including caspase-dependent apoptosis and poly (ADP-ribose) polymerase-mediated DNA repair [5][6][7][8]. With the cost of stroke care in the USA projected to exceed 180 billion dollars by 2030, understanding sex differences and optimizing neuroprotective agents is critical to the development of efficacious therapies [9][10][11].

Macroautophagy (called autophagy thereafter) is a self-catabolic process where subcellular proteins, macromolecules, and organelles are sequestered within membrane-enclosed vesicles (autophagosomes) and are degraded by fusion with lysosomes (autolysosomes) [12][13][14][15]. Autophagy plays a role in cellular homeostasis by degrading damaged cellular contents and redistributing the constituents for other cellular processes [14]. During times of cell stress, such as ischemia, autophagy may become dysregulated and increase injury, or conversely may increase the ability of the cell to survive under conditions with low energy substrates. There is increasing evidence that autophagy is a sex-dependent process [13][16][17][18].

Recent reports have revealed increased levels of autophagy in experimental models of both hemorrhagic and ischemic stroke [19][20][21][22][23]. Pharmacological inhibition of autophagy in male animals during or up to several hours after experimental stroke reduced tissue death [19][20][24][25][26][27]. Studies in neonates show that females have higher basal levels of autophagy and caspase activation following hypoxic-ischemic injury [28]. We hypothesized that sex differences

are present in autophagy in the ischemic brain, and that down regulating autophagy after experimental ischemic stroke in mice would have differential efficacy in males and females.

## 3. Conclusions

It is well established that neuronal tissue is rapidly lost as stroke progresses. Autophagy plays a major role in the response to cellular stress, and has been implicated in the response to tissue ischemia [29]. Evidence suggests that survival of the ischemic penumbra depends strongly on the interaction between regulators of autophagy and apoptosis [30]. This study provides the first evidence that sex differences in autophagy previously noted in vitro are present in vivo following stroke in adult mice. Five key autophagy proteins, p62, LC3, ATG7, Beclin1, and pULK1, show differential expression in response to cerebral ischemia in males and females. Furthermore, post-stroke treatment with 3MA reduced tissue death in males but had no benefit in females, and exacerbated injury. This has important implications for the development of autophagy modulators as neuroprotective agents.

Down regulation of autophagy by 3MA occurs through interaction with a complex consisting of Vps34, p150, and Beclin1 [31]. A recent systematic review analyzed the effects of 3MA on animal models of cerebral infarction [32]. Unfortunately, there is no consensus about the beneficial effects of 3MA on cell viability in the brain: some studies found that inhibiting autophagy with 3MA prevents cell death after ischemic stroke [27][33]; however, other studies propose that 3MA may promote neuronal death [34][35]. However, none of these examined sex-specific effects or were performed only with male animals. Our study provides evidence about sex differences in brain damage and autophagy after ischemic stroke in mice. We found that at 6 h, there were higher levels of Beclin1 in males when compared to females; however, there was no effect of stroke. At 24 h, stroke males had a significant increase in Beclin1 compared to male shams, whereas stroke females had a significant decrease in Beclin1 compared to female shams. The increase in Beclin1 and LC3-II seen in males suggests that males rapidly induce autophagy after ischemia and could explain the selective effectiveness of 3MA treatment in males after stroke. Coinciding with an increase in Beclin1 and LC3-II levels, male mice subjected to stroke had a decrease in levels of p62, suggesting that the autophagy cargo p62 is degraded and autophagy is stimulated. Further, this suggestion is supported by reduced phosphorylation of ULK1 at SER 757, which is phosphorylated by mTOR and is an indicator of enhanced autophagy [36][37]. The number of studies on sex differences in autophagy in animal models of ischemic stroke is limited. To our knowledge, only one study examined sex differences in autophagy, which examined the autophagy regulator, HIF-1α, which was upregulated in male rats 24 h after ischemic stroke compared with females [38]. Thus, our study provides novel data and highlights the importance of studying the mechanisms that govern sex differences in autophagy in the brain after stroke.

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