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NECROSTATIN-1 PROMOTES HEMATOMA RESOLUTION AND ATTENUATES CEREBRAL EDEMA FOLLOWING INTRACEREBRAL HEMORRHAGE

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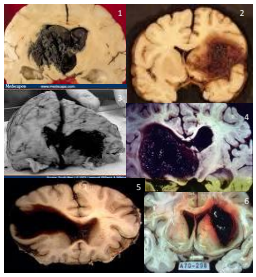
Intracerebral Hemorrhage (ICH)

- Most prevalent type of hemorrhagic stroke
- Caused by the rupture of a diseased blood vessel
- Around 50% of all deaths occur within the first 48 hours
- 50-60% mortality within the first year
- Only 20% regain functional independence



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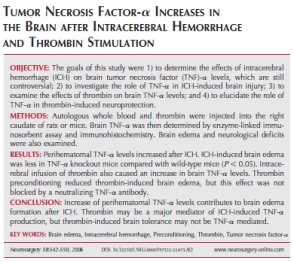
1) neuropathology.neoucom.edu/chapter2; 2) medscape.com; 3) uth.tmc.edu; 4) urmc.rochester.edu; 5) kobiljak.msu.edu; 6) pathcuric.swmed.edu

Tumor necrosis factor α (TNF-α)

- Pro-inflammatory cytokine
- Increases neurological injury after ICH
- Activates RIP Kinase (RIPK) to induce necroptosis, a novel form of cell death



TUMOR NECROSIS FACTOR-α INCREASES IN THE BRAIN AFTER INTRACEREBRAL HEMORRHAGE AND THROMBIN STIMULATION

OBJECTIVE: The goals of this study were 1) to determine the effects of intracerebral hemorrhage (ICH) on brain tumor necrosis factor (TNF)-α levels, which are still controversial; 2) to investigate the role of TNF-α in ICH-induced brain injury; 3) to examine the effects of thrombin on brain TNF-α levels; and 4) to elucidate the role of TNF-α in thrombin-induced neuroprotection.

METHODS: Autologous whole blood and thrombin were injected into the right caudate of rats or mice. Brain TNF-α was then determined by enzyme-linked immunosorbent assay and immunohistochemistry. Brain edema and neurological deficits were also examined.

RESULTS: Perihematomal TNF-α levels increased after ICH. ICH-induced brain edema was less in TNF-α knockout mice compared with wild-type mice ($P < 0.05$). Intracerebral infusion of thrombin also caused an increase in brain TNF-α levels. Thrombin preconditioning reduced thrombin-induced brain edema, but this effect was not blocked by a neutralizing TNF-α antibody.

CONCLUSION: Increase of perihematomal TNF-α levels contributes to brain edema formation after ICH. Thrombin may be a major mediator of ICH-induced TNF-α production, but thrombin-induced brain tolerance may not be TNF-α mediated.

KEY WORDS: Brain edema, Intracerebral hemorrhage, Preconditioning, Thrombin, Tumor necrosis factor-α

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Necrostatin-1 (Nec-1)

- Novel allosteric inhibitor of RIP1 kinase
- Limits neurovascular damage in culture models of hemorrhagic injury

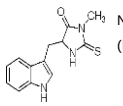
Free Radical Biology & Medicine

Original Contribution

Hemin-induced necroptosis involves glutathione depletion in mouse astrocytes

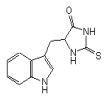
Melissa D. Laird, Chandramohan Wakade, Cargill H. Alleyne Jr, Krishnan M. Dhandapani

- Improve outcome after in pre-clinical TBI and cerebral ischemia models



Necrostatin-1
(EC_{50} = 0.18 μM)

Inactive analog



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Hypothesis

Necrostatin-1 reduces neurological injury after ICH

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Necrostatin-1 Decreases Hematoma Size After ICH

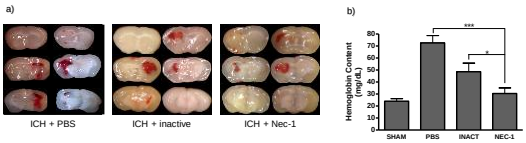

Figure 1. Necrostatin-1 attenuates hematoma size after ICH. Coronal sections from vehicle, inactive analog, or Necrostatin-1 treated animals at 72hr post-ICH (a). Mice treated with either Necrostatin-1 or its inactive analog showed a significant reduction in brain hemoglobin content, a validated measure of hematoma size (b). Data are expressed as mean ± SEM. (*p<0.05, ***p<0.001).

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Necrostatin-1 Reduces Vasogenic Edema After ICH

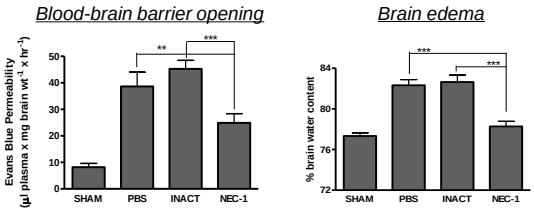

Figure 2. Necrostatin-1 helps maintain blood-brain barrier permeability after ICH. Evans blue dye extravasation was significantly reduced at 3hr post-ICH in animals treated with Necrostatin-1. Data are expressed as mean ± SEM (**p<0.01, ***p<0.001, n=7-9 per group).

Fig. 3. ICH related brain edema is reduced in animals treated with Nec-1. *Brain water content was significantly lower at 24h in animals receiving the active compound. Data are expressed as mean ± SEM (*p<0.05, **p<0.01, ***p<0.001, n=7-9 per group for A and B, n=3-8 for C).*

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Necrostatin-1 Improves Neurological Outcomes After ICH

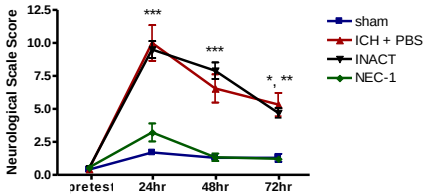

Fig. 5. Nec-1 improves neurological outcome after ICH. *Animals receiving Nec-1 performed better on tests of neurological integrity vs. vehicle. Graph depicts mean ± SEM (*p<0.05, **p<0.01, ***p<0.001; n=9-10 per group).*

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Conclusions

- Necrostatin-1 reduces hematoma size, brain edema, and neurological deficits after ICH

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