Selective inhibition of CPU reduces microvascular thrombosis in experimental rat stroke model

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Only 20% of AIS patients benefit from treatment1,2
- tPA (alteplase)
  • 8-10% of patients eligible
  • 50% recanalization
  • Limited treatment window
  • Hemorrhagic transformation

Mechanical thrombectomy
- 20% of patients eligible
- Highly specialized centres
- Embolization
- 20% un retrievable

Carboxypeptidase U (CPU, TAFI, CPB2) potential target in ischemic stroke
- Potent attenuator of fibrinolysis. 3
- Inactive precursor (proCPU, TAFI, proCPB2) in the blood:
  • Activated by thrombin, thrombin-thrombomodulin and plasmin. 3
  • Very short half-life (8-15 min) due to thermal inactivation (CPU). 3
- CPU inhibitors under development for application in AIS and PE. 4

Might imply ongoing microvascular thrombosis

This after generation saline comparable P< a (A)
- Limited treatment window
- Hemorrhagic transformation

B (B) AZD cleared edema reduced 2010; 403: 114
- Very short half-life
- Potentially reduces downstream microvascular thrombosis

CPU and proCPU determination
- HPLC-based activity assay (Bz-o-cyano-Phe-Arg)1,4

ASSAYS
- tPA (alteplase)
  • Assessment of the kinetic profile of CPU generation in rat experimental stroke models

CONCLUSION
- CPU generation and concomitant proCPU consumption were observed in rat models of acute ischemic stroke
  - Also in saline treated animals: so far not observed in humans
  - Also in a tMCAO model without thrombogenic trigger: Might imply ongoing microvascular thrombosis

There was a reduction of fibrinogen deposition after AZD9684 administration but a significant reduction of brain edema was not observed except after co-administration of tPA: Suggests reduction of microvascular thrombosis by selective CPU inhibition

- Confirmation of occlusion
  - 7-Tesla small animal MRI
- Thrombus injection in MCA
- Thrombus preparation

N=9 in both models

Confirmation of occlusion
Tail vein blood collection
Mca Stenography-Davies rate (320-400 g); N=15
Thrombus preparation
+ thrombin (1 NIH Unit) & CaCl2
Silicone-coated suture (monofilament)

Significant CPU generation upon ischemia induction

CPU generation was observed in all saline treated animals (green) immediately after ischemia induction with peak activity shortly after treatment cessation. CPU generation was even observed in saline treated animals without direct thrombogenic stimulus (tMCAO). tPA administration resulted in higher CPU activities (red) that were comparable with levels observed in humans. In sham operated rats (black), there was no significant CPU generation.

AZD9684 administration results in complete inhibition of CPU activity

The clear CPU generation observed in the tMCAO model after saline (green) or tPA (red) administration was completely inhibited by addition of AZD9684 (purple and blue).

Concomitant proCPU consumption during ischemia induction

Concomitant proCPU consumption was observed with minimal proCPU levels shortly after treatment cessation in both saline (green) and tPA-treated (red) animals. There was a clear upregulation of proCPU at 24 h in animals that were subjected to ischemia that was absent in sham operated rats (black). This might be due to thrombo-inflammation and proCPU being an acute phase protein in rodents.

Selective inhibition of CPU reduces downstream microvascular thrombosis

There was a tendency towards reduced brain edema upon inhibition of the CPU system, but this was only significant in combination with tPA administration (A). CPU inhibition reduced fibrinogen deposition in brain homogenates (B). One-Way ANOVA with Holm Sidak’s multiple comparisons test. * P<0.05

A. Brain edema
B. Fibrinogen deposition