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

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Opening new horizons

CONTEXT

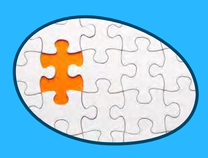
Only 20% of AIS patients benefit from treatment[1,2]

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% unretrievable



Carboxypeptidase U (CPU, TAFIa, 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_i). [3]
- CPU inhibitors under development for application in AIS and PE.[4]

AIMS

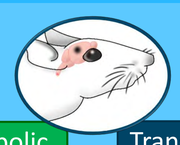
eMCAO and tMCAO

- Assessment of the kinetic profile of CPU generation in rat experimental stroke models

tMCAO only

- Assessment of CPU inhibitor AZD9684
  - Influence on CPU kinetics?
  - Influence on microvascular thrombosis and stroke parameters?

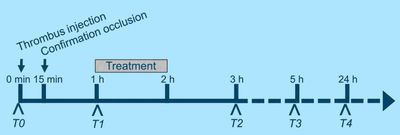
STUDY DESIGN

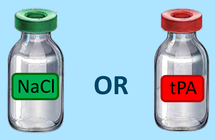


Embolic MCAO[5] | Transient MCAO[6]

Sham
N=9 in both models

Treatment administration and sample collection in eMCAO





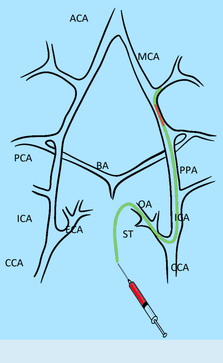
N=17 0.9% m/v | **N=16** 10 mg/kg

CPU and proCPU determination
HPLC-based activity assay
(Bz-*o*-cyano-Phe-Arg)[7,8]

Confirmation of occlusion

7-Tesla small animal MRI

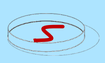
Thrombus injection in MCA



Tail vein blood collection

Male Sprague-Dawley rats (320-400 g); N=33

Thrombus preparation



+ thrombin (1 NIH Unit) & $CaCl_2$
24 h @ 37°C

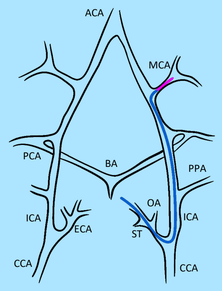
Insertion of monofilament

Male Sprague-Dawley rats (320-400 g); N=34

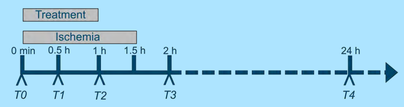
\+

Silicone-coated suture (monofilament)

Occlusion of base of MCA



Treatment administration and sample collection in tMCAO

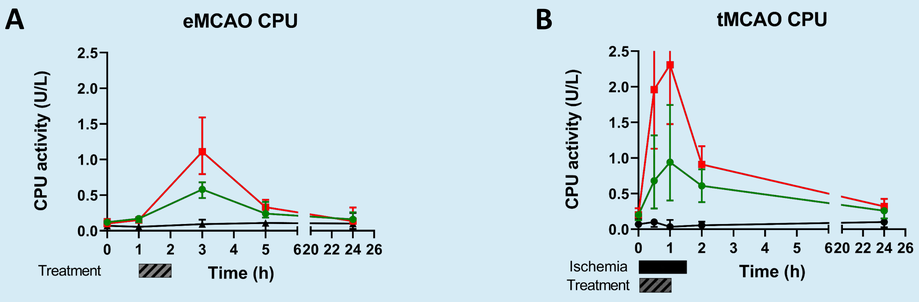


N=9 0.9% m/v | **N=9** 10 mg/kg | **N=8** 9 mg/kg | **N=8** 10 mg/kg + 9 mg/kg

CPU and proCPU determination
HPLC-based activity assay
(Bz-*o*-cyano-Phe-Arg)[7,8]

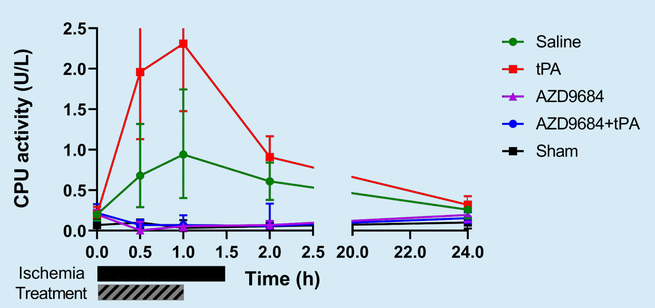
RESULTS

Significant CPU generation upon ischemia induction



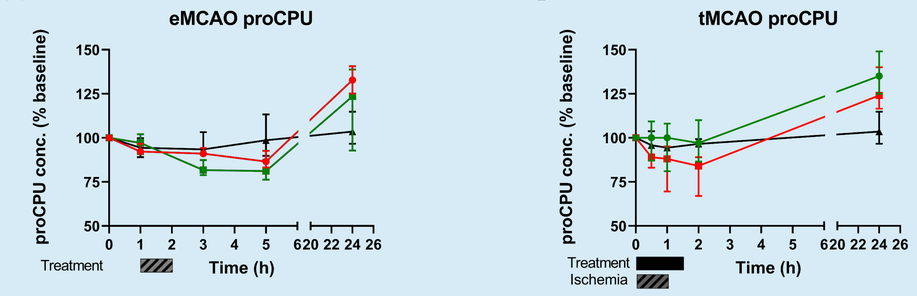
CPU generation was observed in **all saline treated** animals (green) **immediately after ischemia** induction with **peak** activity **shortly after treatment cessation**. CPU generation was even observed in saline treated animals **without direct thrombogenic stimulus** (tMCAO). tPA administration 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



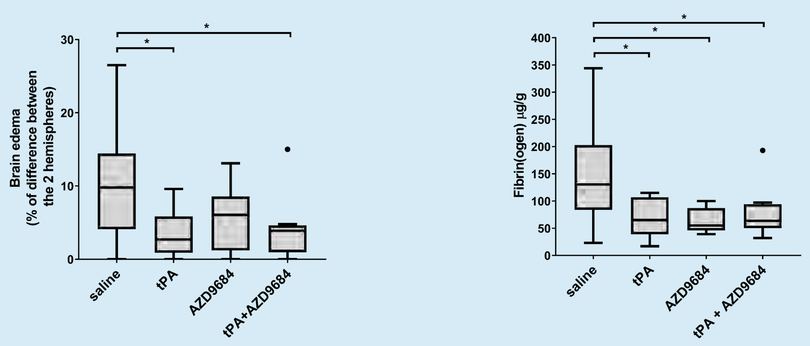
The clear CPU generation observed in the tMCAO model after saline (green) or tPA (red) administration was **completely inhibited by addition of AZD9684** (purple and blue).

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 t**hrombo-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 depositions** in brain homogenates (B). One-Way ANOVA with Holm Sidak's multiple comparisons test. *: P<0.05

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**

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2. **Mokin *et al.*** J NeuroInt. Surg. 2019; 11: 215–20
3. **Leurs *et al.*** Thromb Haemost. 2005; 94: 471–87.
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5. **Lapergue et al.** Stroke. 2013; 44: 699-707
6. **Desilles et al.** Stroke. 2015; 46: 3241-8
7. **Heylen *et al.*** Anal Biochem. 2010; 396(1): 152–4.
8. **Heylen *et al.*** Anal Biochem 2010; 403: 114–6.