

Short communication

## C1-esterase inhibitor reduces infarct volume after cortical vein occlusion

A. Heimann, T. Takeshima, G. Horstick, O. Kempfski \*

*Institute for Neurosurgical Pathophysiology, Johannes Gutenberg-University Mainz, 55101 Mainz, Germany*

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### Abstract

In order to clarify the role of complement as a mediator of cerebral infarct growth, we inhibited the classical complement activation pathway in a photochemical cortical vein occlusion model. Immediately after occlusion, rats were infused with either 0.9% saline (vehicle), or C1-esterase inhibitor (C1-INH) over 30 min. Regional cerebral blood flow (rCBF) decreased after occlusion, and was about 50% of baseline after 2 h. No difference was noted between experimental groups. Mean arterial blood pressure (MABP) and arterial blood gases were likewise unaffected by the treatment. However, administration of C1-INH had significantly reduced infarct volume by 72%, as evaluated after 5 days survival. Thus, the neuroprotective effect cannot be explained by an improvement of cerebral perfusion but rather by protection of the parenchyma in the penumbra. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Complement; C1-esterase inhibitor; Cerebral blood flow; Cortical vein occlusion; Rat

The activation of the complement system may occur by the classical, the alternative, or by the mannose binding pathway. Notwithstanding the trigger, the activation generates the anaphylatoxins C3a and C5a and the terminal components C6–C9 resulting in the terminal complement complex or membrane attack complex (MAC). MAC found in the border zone of myocardial infarction suggests that complement is involved in infarction, which receives further support by the observation that inhibition of the classical complement pathway significantly reduces infarct volume [5]. Recent literature suggests that complement is involved in neurodegenerative diseases, cerebral trauma, and ischemia/reperfusion injury [1,2,7]. The fact that complement is one of the major mechanisms in inflammation, and that it promotes neutrophil adherence to endothelium [10] led us to hypothesize that it might play a central role in cerebral ischemia. Therefore, we used C1-esterase inhibitor (C1-INH) in a model of cortical venous infarction [8], which induces a reproducible cortical infarct, and

allows to accurately monitor therapeutic effects on the comparatively large surrounding zone with critically reduced blood flow. The efficacy of C1-INH was studied by morphometry of the resulting infarct 5 days after two vein occlusions.

Sixteen male Wistar rats ( $317 \pm 44$  g; Charles River, Germany) were randomized to intraarterial treatment with either purified human C1-INH (20 IU/kg b.wt Berinert, Centeon, Germany) or physiological saline (vehicle). All experiments were carried out under anaesthesia (0.5 mg atropine, 36 mg chloral hydrate/100 g b.wt i.p.), spontaneous ventilation, and controlled body temperature ( $37^{\circ}\text{C}$ ) in accordance with German animal protection legislation. Data are reported as means  $\pm$  standard error (S.E.M.). Differences between the groups were determined by *t*-test, within groups by analyses of variance (ANOVA) followed by the Student–Newman–Keuls test (SIGMASTAT, Jandel Scientific, Germany).

Regional cerebral blood flow (rCBF) was assessed by laser Doppler (LD; Vasamedics, USA, 0.8 mm needle probe) scanning in a  $4 \times 5$  mm large parietal cranial window, which had its fronto-medial corner 1 mm lateral and 1 mm caudal to the bregma (dura intact) [4,8]. Under baseline conditions rCBF, obtained from 36 cortical locations, was  $39.4 \pm 6$  LD units (LDU) in the vehicle and  $40.0 \pm 7$  LDU in the C1-INH group (Fig. 1). Mean arterial

\* Corresponding author. Fax: +49-6131-176640; E-mail: kempfski@nc-patho.klinik.uni-mainz.de; <http://www.uni-mainz.de/fb/medizin/nc-patho/>

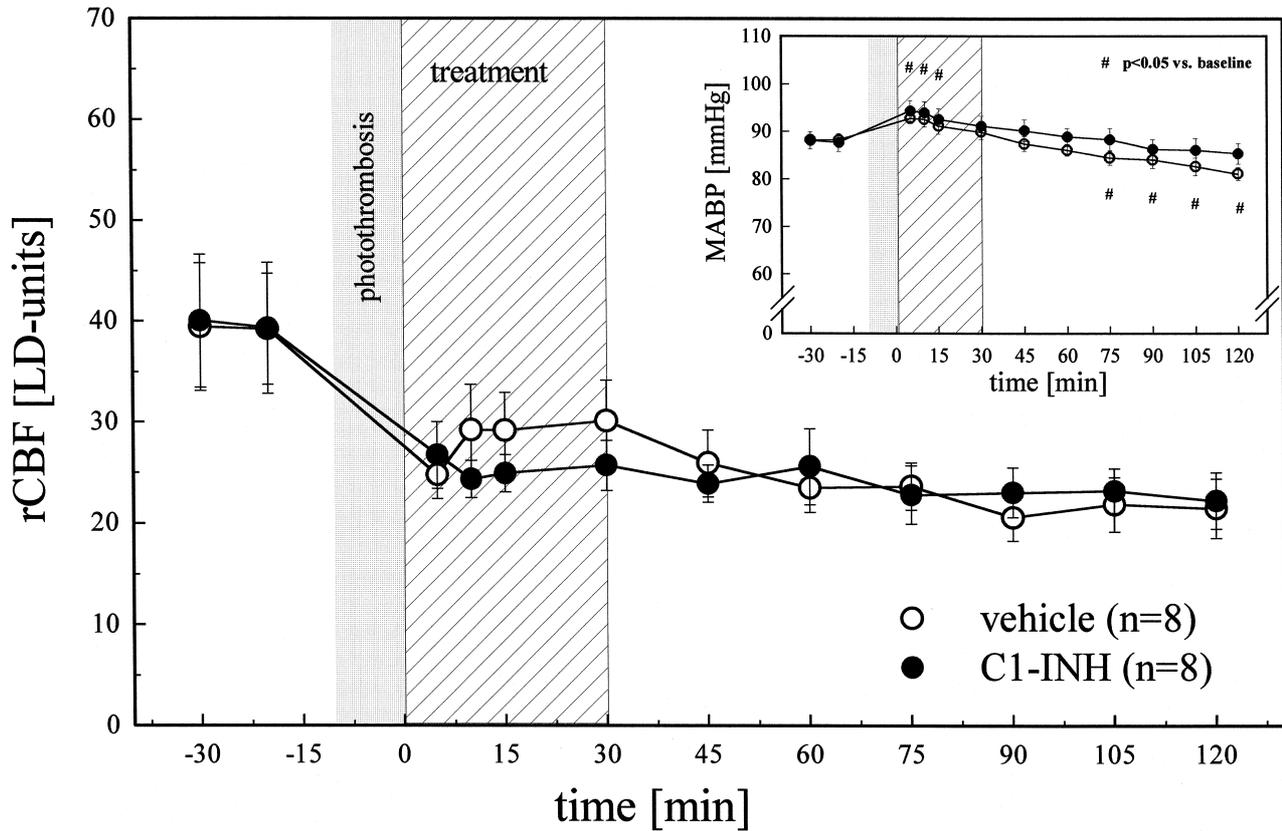


Fig. 1. Time course of rCBF measured by LD scanning in the two experimental groups (means of medians determined from 36 local measurements). Therapy was immediately initiated after thrombosis. The photothrombosis of two cortical veins induced a critical flow reduction which aggravated with time. However, there was no difference in rCBF or MABP (inset) between the both groups.

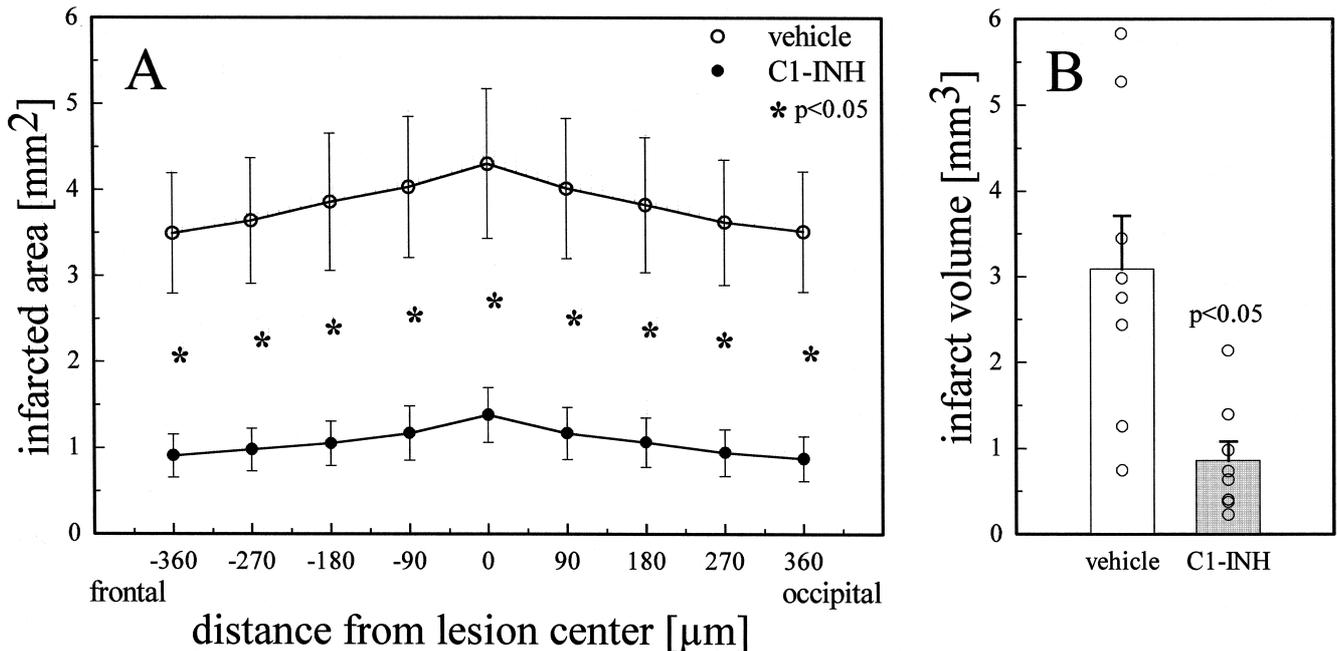


Fig. 2. Brain damage as found 5 days after venous occlusion. Panel A demonstrates the spatial extent of the cortical infarcts in both groups. The resulting infarct volumes are compared in panel B. Symbols represent individual animals. Complement inhibition by C1-INH reduced infarct by 72%.

blood pressure (MABP; baseline:  $88.2 \pm 1$  mmHg vehicle;  $88.1 \pm 2$  mmHg C1-INH) was measured in the proximal section of the carotid artery (Gould, USA). In order to occlude two adjacent cortical veins the photosensitizing dye Rose Bengal was slowly infused (50 mg/kg b.wt) into the femoral vein. Veins were selectively illuminated for 10 min via a micromanipulator assisted light guide (200  $\mu$ m fibre, 540 nm, 5000–6000 lx; for detail see Ref. [8]). Immediately after photothrombosis either 0.5 ml C1-INH (20 IU/kg b.wt) or 0.9% saline were slowly infused over 30 min into the carotid artery. During the first 15 min after venous occlusion a slight transient increase of MABP was observed (Fig. 1; inset). MABP raised maximal to  $93 \pm 1$  mmHg (ns) in the vehicle, and to  $94 \pm 2$  mmHg in the C1-INH group 5 min after photothrombosis ( $p < 0.05$  vs. baseline). There was no statistical difference between the experimental groups. At the same time rCBF was reduced by 40% in the vehicle ( $24.7 \pm 2$  LDU), and by 33% in the C1-INH group ( $26.7 \pm 3$  LDU), respectively. After these initial changes MABP decreased slightly towards the end of observation time in both groups. Two hours after venous occlusion rCBF had identically decreased to approximately 50% of baseline in both groups ( $22.5 \pm 3$  LDU vehicle;  $22.2 \pm 3$  LDU C1-INH). Although there was no difference at any time between the experimental groups neither in MABP nor in rCBF, C1-INH treatment had a dramatic effect on histological outcome. Five days after the experiment the animals were subjected to perfusion fixation (4% paraformaldehyde). Coronal sections (3  $\mu$ m) were stained with haematoxylin/eosin. Histo-morphometry was done in brain slices with the maximally infarcted slice centered. Infarction size on adjacent slices was evaluated in 90  $\mu$ m steps. Infarction volume  $V_I$  was calculated from infarcted areas  $A_n$  and the distance between sections ( $d = 90 \mu$ m) according to the formula:

$$V_I = \sum A_n d.$$

Infarct volume after complement inhibition ( $0.86 \pm 0.23$  mm<sup>3</sup>) was only 27.7% ( $p < 0.05$ ) of that of the vehicle group ( $3.09 \pm 0.62$  mm<sup>3</sup>). Likewise, the infarcted areas as determined in matched slices were significantly smaller in the C1-INH group than in the vehicle group (Fig. 2).

Our main finding is a profound neuroprotective effect of C1-INH treatment in cortical vein thrombosis. C1-INH does not influence hemodynamic parameters, blood gases (data not shown) and microcirculation. Thus, the neuroprotective effect is probably due to a direct protection of the parenchyma, and cannot be explained by amelioration of cerebral perfusion. The photochemical cortical vein thrombosis model induces a slowly growing thrombus going along with a gradually progressing significant reduction of rCBF and a reproducible infarction volume [8]. In comparison to arterial occlusion blood drains depending on the available collaterals and the outflow resistance, causing low blood flow rates, and even retrograde perfusion of tissue sections with desaturated blood. Therefore, tissue

perfusion is reduced rather homogeneously and has characteristics of the ischemic penumbra [3]. As shown earlier for other organs [6] complement may be an important mediator of traumatic and ischemic brain damage. The definite triggers of complement activation in cerebral ischemia/reperfusion, however, are still to be determined. It is now generally accepted, that brain cells can generate a complete, functional complement system, which, once activated, can contribute to damage of glia and neurons [7]. Characteristics of neuronal death after complement activation have been characterized in vitro [9]. Neuroprotection after early C1-INH treatment suggests a major role of plasma complement components in this model, e.g., as in myocardial ischemia where plasma C3a was elevated in early reperfusion [5]. Parenchymal complement constituents may be inhibited only at later stages of infarct development, when blood–brain barrier already is disrupted and C1-INH can reach the neuropil.

In conclusion, the early inhibition of the classical complement activation pathway with C1-INH may be helpful to protect the penumbra zone in focal cerebral ischemia and human stroke. Further studies are necessary to elucidate the optimal time window remaining for complement inhibition after stroke.

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