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Enhanced cortical reperfusion protects coagulation factor XII-deficient mice from ischemic stroke as revealed by high-field MRI

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ABSTRACT

Intrinsic coagulation factor XII deficient ($FXII^{-/-}$) mice are protected from ischemic stroke. To elucidate underlying mechanisms we investigated the early ischemic period *in vivo* by multimodal magnetic resonance imaging (MRI) at 17.6 Tesla.

Cerebral ischemia was induced by either **t**ransient (60 min) or ***p***ermanent occlusion of the middle cerebral artery (***t***/***p***MCAO). 10 $FXII^{-/-}$ mice underwent **t-** , 10 $FXII^{-/-}$ mice ***p-*** and 10 Wildtype (Wt) mice ***t***MCAO. Cerebral blood flow (CBF), diffusion-weighted-imaging (DWI) and T2-relaxometry were measured at 2 h and 24 h after MCAO. Outcome measures were evaluated after motion correction and normalization to atlas space. 2 h after ***t***MCAO CBF reduction was similar in $FXII^{-/-}$ and Wt mice extending over cortical (CBF (ml/100 g/min) 33.6 ± 6.9 vs. 35.3 ± 4.6, $p = 0.42$) and subcortical regions (25.7 ± 4.5 vs. 31.6 ± 4.0, $p = 0.17$). At 24 h, recovery of cortical CBF by +36% was observed only in ***t***MCAO $FXII^{-/-}$ mice contrasting a further decrease of –30% in Wt mice after ***t***MCAO ($p = 0.02$, $F_{(1,18)} = 6.24$). In $FXII^{-/-}$ mice in which patency of the MCA was not restored (***p***MCAO) a further decrease of −75% was observed. Cortical reperfusion in ***t***MCAO $FXII^{-/-}$ mice was related to a lower risk of infarction of 59% vs. 93% in Wt mice ($p = 0.04$). Subcortical CBF was similarly decreased in both ***t***MCAO groups (Wt and $FXII^{-/-}$) relating to a similar risk of infarction of 89% (Wt) vs. 99% ($FXII^{-/-}$, $p = 0.17$).

Deficiency of FXII allows neocortical reperfusion after ***t***MCAO and rescues brain tissue by this mechanism. This study supports the concept of FXII as a promising new target for stroke prevention and therapy.

Introduction

Thromboembolic occlusion of cerebral vessels accounts for about two thirds of ischemic strokes representing one of the leading causes of mortality and chronic disability worldwide (Murray and Lopez, 1997). In acute human thromboembolic stroke rapid recanalization of major arteries represents the principal goal of treatment (Davis et al., 2008). However, it is an unfortunate clinical observation that despite reopening of a previously occluded major cerebral artery, referred to as "recanalization," further infarct growth may occur (Coutts and Goyal, 2009; Soares et al., 2009; Yoo et al., 2009). This may be due to distal thrombus migration or reocclusion, but also occurs in vascular territories of persistently reopened major cerebral arteries. In experimental observations in the stroke model of transient middle cerebral artery occlusion (***t***MCAO), microvascular obstruction, also termed focal "no-reflow," early after the ischemic stimulus is related to the failure of efficient brain tissue reperfusion despite recanalization of major intracerebral branches. Among other important mechanisms, the plasmatic coagulation system (Okada et al., 1994) and activation of platelets (Choudhri et al., 1998) mediate microvascular obstruction and may be potentially important secondary steps in stroke development (reviewed in (Stoll et al., 2008)), which, however, remains to be proven in human stroke.

We have recently shown that coagulation factor XII (FXII; Hageman factor) is involved in thrombus formation after transient middle cerebral artery occlusion (***t***MCAO), the most widely used stroke model (Kleinschnitz et al., 2006). For initiating plasmatic coagulation two distinct pathways exist, either triggered by vessel wall (extrinsic) or by blood-borne (intrinsic) factors, and converge on a common pathway leading to thrombin and fibrin formation. The intrinsic

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pathway of coagulation is initiated when FXII comes into contact with negatively charged surfaces (contact activation) and plays a central role in pathological thrombus formation in various vessel injury models. Importantly, FXII is dispensable for hemostasis after tissue injury (Renne et al., 2005). In the ***t***MCAO stroke model FXII-deficient ($FXII^{-/-}$) mice developed smaller infarcts as revealed by histology (Kleinschnitz et al., 2006) but the precise mechanisms of this stroke protection are unknown. To further address this important functional issue we employed a multimodal MRI protocol at high-field strength of 17.6 Tesla, and followed the early phase of cerebral ischemia and the evolution of infarctions *in vivo* in individual animals over time. $FXII^{-/-}$ mice underwent transient (***t***) or permanent (***p***) MCAO. MRI outcome measures were chosen to characterize cerebral perfusion, hypoxic diffusion restriction by diffusion weighted imaging (DWI), and irreversible infarction as assessed by lesion extent on T2-weighted MRI. Cerebral perfusion as the principal outcome measure was determined as cerebral blood flow (CBF) in the brain microvasculature using water as a freely diffusible tracer by a modified continuous arterial spin labeling (CASL) method (Detre et al., 1992; Williams et al., 1992; Wong et al., 1998). For an exact spatial allocation of all outcome measures to relevant anatomical structures in the mouse brain image registration was performed to a standard mouse brain atlas space. To match responses over time and between groups with sufficient anatomical detail a large sample size was obtained.

With this design we for the first time followed stroke development *in vivo* in a transgenic mouse model using $FXII^{-/-}$ mice by multimodal high field MRI. Our data indicate that CBF is similarly reduced in $FXII^{-/-}$ and Wildtype (Wt) mice early after ***t***MCAO, but recovers in the cortex of $FXII^{-/-}$ animals at later time points leading to smaller infarctions. These findings provide important insights into thrombus formation after experimental stroke and indicate that enhanced tissue reperfusion is one major mechanism that protects $FXII^{-/-}$ mice from ischemic stroke. Importantly, because targeting FXII seems not to be related to an increased risk of bleeding complications, it seems promising in human ischemic stroke for the prevention of postischemic tissue damage.

Materials and methods

Experimental design and animal stroke model

All procedures and animal studies were approved by the Regierung von Unterfranken (Würzburg, Germany) and conducted in accordance with the recommendations for the performance of basic experimental stroke studies as previously published (Dirnagl, 2006). The generation of $FXII^{-/-}$ mice has been described in detail previously (Pauer et al., 2004).

The experimental group in this study were $FXII^{-/-}$ mice undergoing 60 min. occlusion of the MCA (***t***MCAO $FXII^{-/-}$; $N=10$). To control for the effect of $FXII^{-/-}$ deficiency Wt controls (6- to 8-week-old male SV 129 mice, Harlan Winkelmann, Germany) were subjected to the same experimental procedure (***t***MCAO Wt; $N=10$). In addition, another group of $FXII^{-/-}$ mice was exposed to ***p***MCAO, in which the patency of the MCA was not restored (no withdrawal of the intraluminal thread; ***p***MCAO $FXII^{-/-}$, $N=10$). $N=3$ Wt mice underwent sham operation.

The experimental procedures were performed as described in detail previously (Kleinschnitz et al., 2007; Kleinschnitz et al., 2006; Varga-Szabo et al., 2008). Briefly, a standardized suture coated with silicon rubber (6021PK10; Doccol Company, Redlands, CA, USA) was introduced into the right common carotid artery and advanced over the internal carotid artery to the origin of the MCA. The suture was fixed and left in situ and animals were allowed to recover. Operation time per animal did not exceed 15 minutes. After 60 min. animals were re-anesthetized and the suture was withdrawn to allow tissue reperfusion (***t***MCAO). Laser-Doppler flowmetry (Moor Instruments, Devon, U.K.) was used to monitor regional cerebral blood flow (rCBF) in the MCA territory (6 mm lateral and 2 mm posterior from bregma) of wild-type (Wt) and $FXII^{-/-}$ mice ($n=4$/group) (Connolly et al., 1996) (Supplementary Fig. 1). After advancing the thread the decrease in rCBF was similar in both groups indicating sufficient occlusion of the MCA (9.0 ± 2.0% of baseline level in Wt mice versus 8.3 ± 1.5% of baseline level in $FXII^{-/-}$ mice, $p>0.05$). Five minutes after reperfusion rCBF was reconstituted to ~75% of baseline levels and again did not significantly differ between Wt and FXII-deficient mice ($p>0.05$) (Supplementary Fig. 1) documenting that stable restoration of blood flow in the MCA territory can be expected equally for $FXII^{-/-}$ and Wt mice. For ***p***MCAO, the thread was left within the vessel until the end of the experiments at day 1. Sham operation included preparation of the ACC and ligation of its branches without thread insertion. The operations were performed under inhalation anesthesia (2.0% isoflurane in a 70%/30% N_2O/O_2 mixture) and the body temperature was maintained at 37 °C using a servo-controlled heating pad. The anatomy of the cerebral vasculature (Circle of Willis) as well as basic physiological parameters of $FXII^{-/-}$ and Wt mice including blood gases and blood pressure have already been extensively assessed previously and major differences have been excluded (Kleinschnitz et al., 2006; Renne et al., 2005). All subjects were subsequently followed *in vivo* by serial multimodal high-field MRI at 2 h and 24 h.

Multimodal MRI of experimental cerebral ischemia in vivo

Measurement of cerebral blood flow

Cerebral perfusion was measured using a modified single coil continuous arterial spin labeling (CASL) method in combination with a turbo spin-echo imaging sequence (Detre et al., 1992; Williams et al., 1992; Wong et al., 1998). To benefit especially from increased longitudinal magnetization and the elevation of the T1 relaxation time all measurements were performed at ultra-high field strength (17.6 T, 750 Hz, Biospin, Bruker BioSpin GmbH, Ettlingen, Germany). The inner diameter of the vertical wide-bore magnet was 89 mm. The employed gradient system had a strength of 200 mT/m and was combined with an in-house built transmit/receive linear 38 mm diameter birdcage resonator. The RF pulse used for adiabatic inversion of arterial spins flowing through the neck was of 2 s duration, 11.6 μT power and 17.8 kHz offset (1.4 cm distance from imaging plane, due to a tagging-gradient of 29.8 mT/m). The control image was acquired by reversing the sign of the tagging gradient to compensate for magnetization transfer effects. Image maps of cerebral perfusion were calculated on a pixel-by-pixel basis according to the equation (Detre et al., 1992):

$$\mathrm{CBF} = \mathrm{lambda}/(2\ \mathrm{alpha}\ \mathrm{T1})\ \exp(-\mathrm{dt}/\mathrm{T1})(\mathrm{M(ctrl)}-\mathrm{M(inv)})/\mathrm{M(ctr)}$$

with M(ctrl) as the signal intensity of the control image and M(inv) as the signal intensity of the tagged image. The degree of the inversion efficiency was assumed to be alpha = 0.7 (Maccotta et al., 1997; Zhang et al., 1993), and the brain-blood partition coefficient value for water lambda = 0.95 mL/g (Herscovitch and Raichle, 1985). T1 denotes the apparent tissue longitudinal relaxation time and dt a delay time of 500 ms.

Parameters for the fast spin-echo imaging sequence (RARE) were: echo train length (ETL) = 8, effective echo time $TE_{eff}=17.2$ ms, repetition time TR = 1 s, slice thickness 1.5 mm, FOV 2.5 × 2.5 cm, matrix of 64 × 64 voxels. The signal was averaged over 12 repetitions resulting in a total acquisition time of 9.5 min.

Slice selective T1 (T1s) mapping

T1s weighted imaging was measured using a single slice partial saturation inversion recovery RARE sequence of eight variable inversion delays (0.02 s, 0.5 s, 1.0 s, 1.5 s, 2.0 s, 3.0 s, 5.0 s, 10.0 s) and a constant recovery time after image acquisition of 10 s, ETL = 16,