Tumor necrosis factor-alpha (TNF-α) can activate luminal endothelium in intraparenchymal segments of the cerebral vasculature and predispose those segments to thrombosis. Spontaneously hypertensive rats have increased numbers of perivascular macrophages around their intraparenchymal brain vessels and release increased amounts of TNF-α in response to lipopolysaccharide (LPS) and also develop more strokes after LPS administration than normotensive rats. In addition, normotensive Sprague Dawley rats can be made susceptible to strokes induced by LPS by activating their macrophages with Bacillus Calmette Guerin. Two weeks after injection of these organisms, the macrophages are highly activated and a dose of LPS intracisternally causes small vessel brain infarcts. These infarcts can be completely prevented by administration of TNF-binding protein (TNFbp) at the time of the LPS infusion.

TNF-α is acutely expressed following focal cerebral ischemia and blocking its activity with TNFbp has been demonstrated to reduce infarct volume in several models of focal ischemia. In permanent middle cerebral artery occlusion (MCAO) in the SHR the evolution of infarction during the first four hours of ischemia was reduced by 34-38% and this neuroprotection was associated with preservation of microcirculatory perfusion suggesting that vascular responses to TNF-α contribute to its pathological action. In BALB/C mice subjected to permanent MCAO, TNFbp superfused onto ischemic brain through the craniotomy opening also reduced infarct volume to a significant degree and compared favorably with other experimental therapies indicating that TNF-α participates in secondary brain injury during the early hours of ischemia.

Evidence has been developed that TNF-α is involved in the signaling pathways that regulate the development of tolerance. Initial studies in a permanent MCAO model in the SHR demonstrated that LPS, a prototypic stimulus for cytokine release, could precondition animals such that they became tolerant to subsequent MCAO. TNFbp completely nullified the capacity of LPS to induce this tolerance. TNF-α was also demonstrated to be sufficient to induce tolerance to MCAO in mice. Although tolerance is generally attributed to an increase in neuronal resistance to ischemia, studies of the microcirculation after LPS administration in
SHR revealed that preservation of microcirculatory perfusion is a feature of the tolerant state. Primary cultures of adult brain microvessel endothelial cells and neonatal astrocytes and neurons have provided direct evidence that TNF-α is involved in the signaling pathways that regulate cellular tolerance.
Activation of Factor IX by Erythrocyte Membrane and Its Possible Significance in Thrombus Formation at Stasis

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It has been recognized that abnormalities such as an enhanced erythrocyte aggregability, an elevated hematocrit and a high blood viscosity at stasis might be causative factors in intravascular coagulation. However, there is no enough evidence to conclude that these factors stimulate the coagulation. In our previous study using an *in vitro* rheological system simulating a stagnant flow condition, we postulated that either factor IX or factor X is activated by erythrocyte membrane, where platelets and leukocytes were not incorporated in initiating the coagulation cascade. We show evidence that human erythrocyte can activate factor IX in the presence of calcium ions, leading to the generation of thrombin. We attempted to purify factor-IX activating protein in human erythrocyte membrane. The rate of activation of factor IX was enhanced by the formation of erythrocyte aggregates and by the elevation of hematocrit. The rate of activation of factor IX by erythrocyte membrane from blood with thrombotic tendencies was much faster than that from normal subject. The intrinsic coagulation pathway initiated through the activation of factor IX by erythrocyte membrane may significantly contribute to the local thrombus formation in areas of retarded blood flow when the delicate balance between regulating hemostasis and avoiding thrombosis is lost.
Intraischemic Hypothermia Attenuates Intercellular Adhesion Molecule-1 (ICAM-1) and Migration of Neutrophil Following Transient Focal Ischemia in Rats

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Adhesion of neutrophil to the endothelium and subsequent transmigration has been reported to contribute to progression of focal ischemia. Hypothermia has been known to attenuate ischemic insult through various mechanisms of action. The authors evaluated the effect of hypothermia on expression of intercellular adhesion molecule-1 (ICAM-1) protein and on transmigration of neutrophil with immunohistochemical method. Transient focal ischemia model in rats was employed, and animals received two hours of either normothermic or hypothermic ischemia. To confirm the effectiveness of hypothermia on neuroprotection, infarct area was compared between the two groups. Our result demonstrated that hypothermia reduced both the number of microvessels expressing ICAM-1 (Fig.1) and that of neutrophils migrating into ischemic tissue (Fig.2). Comparison of infarct area showed persistent protective effect in cortex, but not in striatum. This study indicates that reduction of ICAM-1 expression and subsequent reduction of migrating neutrophil in hypothermia can contribute to attenuation of ischemic damage.

Fig.1 : Temporal profile of the number of ICAM-1 positive cells.

Fig.2: The number of MPO positive cells in cortex.
Selective Thrombin Inhibitor (Argatroban) Ameliorates Platelet Adhesion to Human Brain Microvascular Endothelial Cells In Vitro. Observation by VEC-DIC Microscopy

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Thrombin is known to appear in the stagnant blood in ischemic tissue. We previously reported that platelets adhered to thrombin-treated human aortic endothelial cells in vitro using video enhanced contrast-differential interference contrast (VEC-DIC) microscopy (1). The present study examined whether activated platelets adhere to human brain microvascular endothelial cells (HBEC) in vitro and whether argatroban (selective thrombin inhibitor) ameliorates the adhesion of activated platelets to HBEC in vitro.

Methods: VEC microscopy consisted of an inverted Nomarski microscope and a CCD camera coupled with an image processing processor, monitored at video image magnifications up to x12000, and recorded on video tape. Experiment 1 (N=9): HBECs were cultured on a coverglass and put in the observation chamber of VEC-DIC microscopy. Then, platelet rich plasma (PRP), separated from venous blood of healthy volunteers, with ADP (2μM) was superfused with an infusion pump at shear rates (10-50/sec) for 30 min and washed out. Interaction between platelets and endothelial cells was observed by VEC-DIC microscopy and the number of platelets adhered to HBEC was calculated. Experiment 2 (N=9): PRP with ADP (2μM) and argatroban (5μg/ml) was superfused for 30 min and platelet adhesion to HBEC was observed. Experiment 3 (N=5): PRP was superfused for 30 min and platelet adhesion to HBEC was observed. To quantify the degree of platelet adhesion to HBEC, the number of adhering platelets in a field 30 micrometers in length and breadth was counted. One hundred consecutive fields were counted and the average number was calculated.

Results: Experiment 1: Platelets adhered to HBEC in all experiments and microaggregates of platelets were seen. The average number of platelets adhering and aggregating to HBEC was 25.5±11.3/900 μm². Experiment 2: Platelet adhesion to HBEC was rarely seen. The average number of platelets adhering and aggregating to HBEC was 1.8±1.8/900 μm². (p<0.01, vs Experiment 1). Experiment 3: Platelet adhesion to HBEC was rarely seen. The average number of platelets adhering and aggregating to HBEC was 0.3±0.6/900 μm².
Comments: The above results showed argatroban ameliorated adhesion and aggregated pileup of activated platelets to HBEC at a low-now state in vitro. This suggests that thrombin produced by platelet activation makes HBEC procoagulant and is the most likely candidate to subsequently induce platelet adhesion to HBEC. Argatroban is of potential usefulness in preventing progress of microcirculatory derangement in patients with acute cerebral ischemia.

Reference
Magnetic Resonance Imaging Demonstrates That Tissue Plasminogen Activator (rt-PA) Increases Stroke Volume If Cerebral Arteries Are Not Successfully Recanalized

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Background and Purpose: The role of rt-PA during thrombolysis of stroke is poorly understood¹². Recently, a direct neurotoxic effect of rt-PA was reported³. This is at odds with studies that show an improved outcome⁴. However, the effect of rt-PA after failure to recanalize the occluded brain vessel was never assessed. Therefore, we evaluated the contribution of recanalisation and rt-PA-infusion to outcome.

Methods: Sprague-Dawley rats underwent thromboembolic stroke⁴. They were assigned to four groups (Fig.1) according to patency of the middle cerebral artery on cerebral x-ray angiography after treatment with saline (n=7) or rt-PA (n=8) (15mg/kgbw over 90 min, starting 1 hour after embolization): (a) control without recanalisation (n=5), (b) rt-PA treatment without recanalization (n=5), (c) control with spontaneous recanalisation (n=2), and (d) rt-PA treatment with recanalisation (n=3). Six sets of diffusion- and perfusion-weighted (bolus-track) MR images were acquired throughout the 6 h observation period. At 6 h after embolization the brains were perfusion-fixed and further processed for histology.

Results: When recanalization after thromboembolic stroke was successful rt-PA significantly

![Figure 1](image1)

![Figure 2](image2)
improved perfusion values and thus a smaller final infarct size resulted in comparison to non-recanalized animals (Fig.1). However, rt-PA increased infarct volume if recanalization was not achieved. MR diffusion imaging showed a similar lesion volume at 1 h after embolization in non-recanalized rt-PA treated and control animals (34.3±19.8 versus 37.9±9.7), but a significant difference in final infarct volume at 6 h (67.4 ± 5.4 versus 47.7 ± 17.9, p = 0.042). Correlation of overall perfusion values and diffusion changes revealed that treatment with rt-PA causes an offset which results in larger lesion sizes at corresponding perfusion values of control animals (Fig.2).

Conclusions: Our results show that rt-PA induced recanalization surpasses any potentially harmful effects and improves outcome in a model of thromboembolic stroke. In contrary, if recanalization is not successful, the detrimental effects of rt-PA appear to dominate and our results suggest that these detrimental effects are not related to perfusion.

References