

Editorial

# Ischaemic damage of brain microvessels: inherent risks for thrombolytic treatment in stroke

Recent trials of plasminogen activators in acute ischaemic stroke underscore the delicate balance between promise of benefit and risk. Attempts to manage clinical outcome by systemic infusion of recombinant tissue plasminogen activator (rt-PA) have so far had mixed success.<sup>1,2</sup> The benefits seen in the National Institutes of Neurological Diseases and Stroke (NINDS) trial of rt-PA in acute ischaemic stroke were a significant absolute improvement in disability outcome.<sup>2,3</sup> However, the risks in that study and in the European Cooperative Acute Stroke Study (ECASS)<sup>1</sup> included further disability and mortality associated with haemorrhagic consequences of the plasminogen activator (PA).<sup>1,2</sup> Three recent studies of streptokinase in acute ischaemic stroke were terminated because of safety concerns.<sup>4-9</sup> In all five trials, the risk of symptomatic haemorrhage associated with the PA was significantly increased over placebo (table 1). In addition, approaches which effect recanalisation of documented cerebral arterial thrombotic occlusions by intravenous or intra-arterial PA infusion have yet to be rigorously shown to promote overall benefit, although anecdotal evidence suggests that individual patients may improve clinically.<sup>10-24</sup> The reasons are practical. The perceived risks, those of angiography and the interventional procedures required for intra-arterial PA delivery, have not been fully evaluated,<sup>25</sup> although the relative risks of diagnostic angiography are low.<sup>26-28</sup> Clearly, this assessment hides many important differences in PA behaviour, study design and conduct, patient populations, diagnostic and therapeutic procedures, disease severity, comorbidity, and a host of other potential contributors. None the less, concerns which may erode the promise of benefit of thrombolytic agents in acute ischaemic stroke include the accentuation of innate risks associated with the evolution of ischaemic injury after the stroke event itself.<sup>29-34</sup> These include early mortality caused by severe brain oedema and

the development of haemorrhagic transformation which causes clinical deterioration or death.

The nature of these risks and their augmentation by PAs are so far not completely understood. For rt-PAs they seem to encompass the time from onset of ischaemic stroke symptoms to treatment for documented middle cerebral artery (MCA) occlusion,<sup>35</sup> diastolic hypertension,<sup>36</sup> body mass,<sup>36</sup> age,<sup>37</sup> signs of ischaemic injury at baseline,<sup>1</sup> and perhaps other yet to be identified factors. It is clear that a reduction in the frequency of symptomatic intracerebral haemorrhage may substantially improve clinical outcome by reducing one significant contributor to mortality in each of the recent trials.<sup>1,2,4-9,29-32,38</sup> The thesis we consider here is that ischaemic injury to the microvasculature, manifest indirectly by early signs of focal ischaemic injury on CT, is central to the risks of oedema and of parenchymal haemorrhage. Here, we posit that appropriate patients with ischaemic stroke will derive further benefit from PAs if the territory and volume of damaged cerebral microvasculature, and thus the risk of oedema formation and symptomatic haemorrhage, is relatively small.

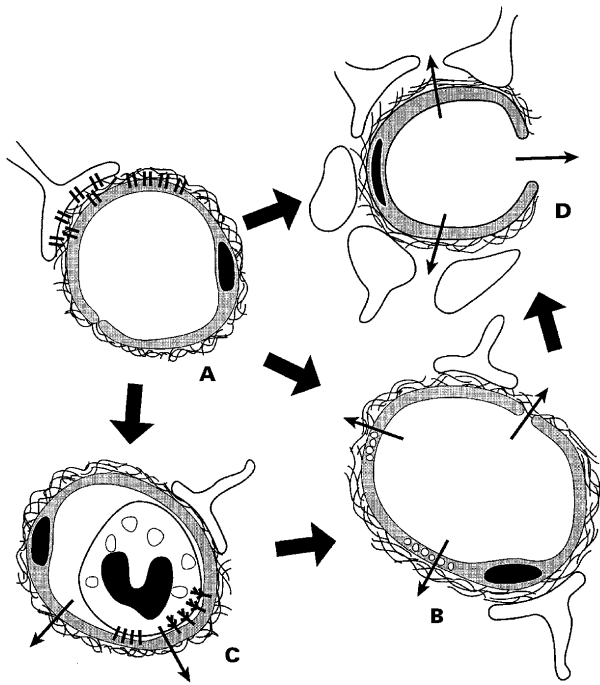
### The microvasculature as a target of ischaemic injury

When blood flow through a feeding cerebral artery ceases, the downstream microvasculature is a proximate target. Although non-vascular cells have long been thought to be the primary target of ischaemic insults, events occurring at the blood-vascular-parenchymal interfaces are necessary for the initiation of tissue injury (figure A-D). During experimental focal cerebral ischaemia, with the fall in tissue oxygenation (1) microvascular permeability barriers are lost (figure B)<sup>39-42</sup>; (2) the microvascular endothelium responds by the sequential expression of leucocyte adhesion receptors (figure C)<sup>43-45</sup>; (3) the basal lamina and

Table 1 Odds ratio (OR) analysis of haemorrhagic transformation in trials of thrombolytic agents in acute ischaemic stroke

Trial	All types of haemorrhages				Symptomatic haemorrhages				Fatal haemorrhages			
	Agent odds	Placebo odds	OR (agent/placebo)	95% CI	Agent odds	Placebo odds	OR (agent/placebo)	95% CI	Agent odds	Placebo odds	OR (agent/placebo)	95% CI
ECASS	134/179	113/194	1.3	0.93-1.77	35/278	9/298	4.2	1.97-8.83	19/294	7/300	2.8	1.15-6.69
NINDS	34/278	11/301	3.3	1.66-6.73	20/292	2/310	10.6	2.46-45.82	9/303	1/311	9.2	1.16-73.36
I+II												
MAST-E	49/88	17/116	3.8	2.04-7.04	24/113	4/129	6.8	2.31-20.34	—	—	—	—
MAST-I	39/118	16/140	2.9	1.54-5.44	10/147	1/155	10.5	1.33-83.40	—	—	—	—
ASK	56/118	28/138	2.3	1.40-3.92	23/151	5/161	4.9	1.82-13.23	17/157	3/163	5.9	1.69-20.47

The response to rt-PA or streptokinase treatment is shown by the OR for different trials. The OR is measured by dividing the odds for a certain outcome event of the rt-PA group (numerator) by the odds for this event of the placebo group (denominator). An OR of 1 means that the thrombolytic agent did not affect the incidence of haemorrhages. If the 95% CI does not cover unity, the incidence of haemorrhages is significantly enhanced or diminished by the treatment. — = not available. In all trials a significant increase in all types of haemorrhages, of symptomatic haemorrhages, and of fatal haemorrhages was found. The NINDS trial showed the highest relative risk for fatal haemorrhages.



Schematic diagram of the effect of ischaemia on microvascular permeability and integrity. (A) Normal cerebral microvessel. Endothelial cells and astrocytes bound to basal lamina by integrin adhesion receptors. Blood-brain barrier intact. (B) Breakdown of blood-brain barrier. (C) Leucocyte adhesion by receptors on endothelium and granulocytes; increased permeability caused by granule release. (D) Breakdown of basal lamina with loss of astrocyte and endothelial cell contacts; permeability to large cells—for example, erythrocytes (see text).

extracellular matrix (ECM) undergo progressive loss of component antigens (figure D)<sup>46</sup>; and (4) cell-matrix adhesion interactions within microvessels are altered (figure D).<sup>47 48</sup>

### Microvascular permeability barriers

The permeability barrier consists of: (1) the blood-brain barrier, represented by the interendothelial cell tight junctions which regulate substrate transfer<sup>39 40</sup>; (2) the basal lamina, a structural barrier to the extravasation of cellular blood elements<sup>49 50</sup>; and (3) perivascular astrocytes which make up the parenchymal component of the microvasculature (figure A).<sup>51</sup> With the loss of the endothelial cell permeability barrier,<sup>39 40</sup> and degradation of basal lamina/ECM components, the brain parenchyma is exposed to the blood plasma and its cellular elements.<sup>39 40 46 51-53</sup>

The formation of the endothelial blood-brain barrier relies on the interdependence of endothelial cells and astrocytes, as elegantly shown in chick-quail adrenal vascular tissue/brain tissue xenograft and fetal-adult hippocampal/neocortex allograft preparations.<sup>54 55</sup> Astrocytes promote microvascular endothelial blood-brain barrier properties including tight junctions, dye exclusion, and antigenic features.<sup>40 56 57</sup> Coculture experiments have shown that soluble factors are necessary to maintain some of those barrier characteristics.<sup>55 58 59</sup>

Intact basal lamina also requires juxtaposition of the microvascular endothelium and astrocytes.<sup>60 61</sup> In culture, astrocytes can secrete the basal lamina/ECM components laminin and fibronectin,<sup>62</sup> as well as chondroitin sulphate proteoglycan.<sup>63</sup> Conversely, collagens stimulate astrocyte induced endothelial cell maturation.<sup>64</sup> Microvascular endothelial cell derived ECM components stimulate astrocyte growth and generation of glutamine synthetase.<sup>65 66</sup>

The integrity of mature cerebral microvessels also seems to involve adhesion receptors which link the cellular components to specific ligands within the basal lamina/ECM.

For instance, it has recently been shown that integrin  $\alpha_1\beta_1$ , a receptor for the ECM ligands laminin-1 and collagen IV, is found on microvascular endothelium,<sup>47</sup> and integrin  $\alpha_6\beta_1$  is expressed on astrocyte end feet juxtaposed to its matrix ligand laminin-5.<sup>48</sup> Hence, development of the blood-brain barrier and the basal lamina depend on the proximity of astrocytes to the endothelium, whereas the integrity of this relation involves cell matrix adhesion molecules.

### Focal ischaemia and changes in microvascular integrity

After experimental MCA occlusion, microvascular expression of integrins  $\alpha_1\beta_1$  and  $\alpha_6\beta_4$  decreases rapidly in the region of focal ischaemia.<sup>47 48</sup> A more gradual loss of laminin-1, laminin-5, cellular fibronectin, and collagen IV antigens within the basal lamina/ECM also occurs.<sup>46 48</sup> Hamann *et al* demonstrated a significant correlation between the development of haemorrhagic transformation and the regional loss of basal lamina architecture by 24 hours after MCA occlusion.<sup>46 52 67</sup> Hence, haemorrhagic transformation occurs in regions where ischaemia causes a breakdown in the basal lamina, and disruption of adhesion between microvascular cellular components and the internal matrix scaffolding.

### Degradation of microvascular barriers

The loss of constituents of the basal lamina and the rapid disappearance of related integrins may reflect down regulation of mRNA or protein synthesis, or degradation of mRNAs or proteins by enzymes secreted by endothelial cells, astrocytes, or circulating cells (figure D). At least three pathways may potentially lead to degradation of components of the basal lamina: (1) activation of plasminogen by endogenous PAs; (2) secretion of metalloproteinases (MMPs); and (3) release of PMN leucocyte specific granule enzymes. Non-ischaemic cerebral tissue generates PA activities.<sup>68</sup> More specifically, cerebral microvascular endothelial cells express t-PA *in situ*<sup>69 70</sup> and *in vitro*,<sup>71</sup> whereas astrocytes produce t-PA and u-PA in response to specific mitogens.<sup>72</sup> Matrix laminins are substrates for plasmin,<sup>73 74</sup> and plasmin activates MMP-1 (collagenase) and MMP-3.<sup>75</sup> Certain MMPs released from vascular endothelium and leucocytes during the inflammatory phase of cerebral ischaemia employ collagen IV (MMP-1, MMP-2, and MMP-9) and laminin (MMP-2 and MMP-9) as substrates to promote tissue remodelling.<sup>76 77</sup> Gelatinases A (MMP-2) and B (MMP-9), which cleave collagen IV, are present in plasma.<sup>78</sup> Rosenberg *et al* have suggested that MMP-9 appears by 24 hours after MCA occlusion in the anaesthetised rat.<sup>79 80</sup> Also, enzymes from PMN leucocyte granules, including collagenase (MMP-8), elastase, and cathepsin G, released during their activation, degrade laminins and collagens.<sup>81-85</sup> It is postulated that these pathways, unopposed by their inhibitors, may contribute to the dissolution of the basal lamina/ECM and the blood-brain barrier, thereby disrupting the normal relations of endothelium to astrocytes.<sup>86 87</sup>

### Leucocytes and microvessel permeability

Circulating leucocytes may promote changes in microvascular permeability at several levels. Adhesion of PMN leucocytes to specific endothelial cell receptors contributes to loss of microvascular patency,<sup>88-91</sup> initiates diapedesis and transit into the parenchyma, and thereby adds to tissue injury (figure C).<sup>92</sup> After MCA occlusion, P-selectin, ICAM-1, and E-selectin are expressed on cerebral microvascular endothelium in a distinct sequence.<sup>43 44</sup> P-selectin responds to thrombin generated during coagulation activation, histamine, activated complement, and superoxides.<sup>93-95</sup> Astrocytes and microglia generate cytokines

which stimulate endothelial cell-leucocyte adhesion receptor expression and promote chemotaxis.<sup>96-98</sup> For instance, ICAM-1 and E-selectin appear in response to interleukin (IL)-1 $\beta$  and tumour necrosis factor (TNF)- $\alpha$ .<sup>99-100</sup> These events initiate and expand the cellular inflammatory response to ischaemic injury. In addition, IL-1 has been shown to directly mediate neuron injury.<sup>96-97</sup> Blockade of IL-1 by exogenous IL-1ra in a rodent MCA occlusion model has been associated with a significant reduction in ischaemic injury at 24 hours.<sup>97</sup> During leucocyte transmigration and activation, the respiratory burst generates superoxide free radicals and release of granule proteolytic substances which contribute to blood-brain barrier, basal lamina/ECM, and perivascular tissue degradation.<sup>101</sup> Cleavage products of laminins, generated by this process and by plasmins, are potent chemoattractants for leucocytes.<sup>102</sup> The presence of complement C5 receptors on astroglial cells<sup>103</sup> suggests the possibility that activated complement generated during ischaemic injury may also contribute to leucocyte activation. Free radicals, localised to ischaemic scattered cerebral microvascular endothelial and smooth muscle cells *in vivo*,<sup>104-106</sup> may increase cerebral endothelial permeability and alter adhesion receptor distribution.<sup>107</sup> In other systems, tissue haemorrhage accompanies tissue injury with inflammation.<sup>108</sup>

#### Barrier loss and haemorrhagic transformation

Extravasation of plasma and blood cells is initiated very early and becomes evident by 24 hours after experimental MCA occlusion in the corpus striatum.<sup>52-67-109</sup> Haemorrhagic transformation seems tied to processes which alter the integrity of the blood-brain barrier and the basal lamina. In clinical preparations and certain experimental models, parenchymal haemorrhage has also been attributed to the impact of systemic blood pressure, the downstream movement of thromboemboli, or rupture of larger injured vessels which may have undergone degenerative changes.<sup>30-110-113</sup> The distinguishing features are the rapid extravasation of blood leading to the compression of contiguous and adjacent tissues with loss of neurological function.<sup>30-110</sup> It is, so far, conjectural whether the processes leading to the development of coagula in the ischaemic zone begin with the microvascular events that lead to haemorrhagic infarction,<sup>33-34-52</sup> however, vascular disintegration would seem to be necessary.

Several well known conditions which are aggravated by advancing age, long term hypertension, and diabetes target the microvasculature and may contribute to loss of microvascular integrity.<sup>114</sup> These include lipohyalinosis or microangiopathy, which has been classified in three states of damage severity:<sup>115</sup> (1) sclerotic and hyalinotic thickening of the vessel wall with onion skinning, which primarily affect arterioles 100–200  $\mu$ m diameter<sup>116</sup>; (2) additional disorganisation of the vessel wall and disruption of the internal elastic lamina with occasional foam cells (these changes may be associated with haematoma formation)<sup>117</sup>; and (3) additional fibrinoid degeneration of the vessel wall with thrombosis.<sup>118</sup> Importantly, deposition of amyloid in the vessel wall (amyloid angiopathy) leads to loss of vascular wall integrity, leakage, and later haemorrhagic changes.<sup>119-123</sup> Most experimental models of focal ischaemia do not mimic these age-dependent vascular disturbances, which may also explain the rare occurrence of large haemorrhages in animal models compared with humans. Hence, loss of microvascular integrity may connect the experimental findings of blood cell extravasation to clinically important haemorrhagic transformation.<sup>35</sup>

#### Oedema formation

During focal cerebral ischaemia, increased microvascular permeability also leads to the entrance of fluid and plasma proteins into the injured region (figure B).<sup>124</sup> In selected models, exposure of coagulation factors to perivascular tissue factor (TF) results in intravascular and extravascular fibrin deposition.<sup>125-126</sup> Here, thrombin is generated which may increase endothelial cell permeability.<sup>127-129</sup> Platelet activating factor (PAF), TNF- $\alpha$ , and bradykinin released during focal ischaemia also increase endothelial permeability.<sup>130-132</sup> Furthermore, tissue haemorrhage itself may promote changes in permeability of the blood-brain barrier and oedema formation.<sup>133</sup>

Brain oedema develops immediately when regional cerebral blood (rCBF) falls below the threshold of structural integrity at 10–15 ml/100 g/min.<sup>124-134-136</sup> In a feline model, within 4 hours of MCA occlusion, cortical water content increased steadily from 80.7% to 83.0% wet weight.<sup>134</sup> This 2.3% net uptake of water was accompanied by increased tissue Na<sup>+</sup> and decreased tissue K<sup>+</sup> concentrations, a shift of water from the extracellular into the intracellular compartment, and a linear increase in brain volume.<sup>134</sup> Fluid entry involves osmotic and ionic gradients between blood and ischaemic brain tissue, and pinocytosis of water in the presence of residual plasma flow.<sup>137-140</sup> Swelling within the ischaemic zone is explained by the failure of energy dependent membrane ion exchange pumps, subsequent influx of Na<sup>+</sup> and water into the intracellular compartment, resulting in cell hydrops at the expense of the size of the extracellular space.<sup>141</sup> In the early stages of experimental focal ischaemic injury, increased transport of plasma markers is associated with increased pinocytosis in brain vascular endothelial cells consistent with attempts by the vasculature to compensate for decreased substrate delivery.<sup>142-143</sup> Here, the ultimate source of the expanded intracellular water compartment is vascular.

In focal ischaemia, the accumulation of tissue water may not resolve if reperfusion is achieved later than one hour after arterial occlusion.<sup>137</sup> Moreover, reperfusion can further enhance oedema in areas of dense ischaemia.<sup>137</sup> Subsequent studies in focal cerebral ischaemia indicate that PMN leucocytes may contribute further to tissue water accumulation in the injured territory.<sup>144-146</sup> Taken from studies of oedema in the skin,<sup>147</sup> it is possible that alterations of adhesive processes that bind neurons to their glial neighbours may further promote oedema formation.

#### Oedema formation and early radiological signs of ischaemia

The net uptake of water by the ischaemic brain tissue affects x ray attenuation.<sup>148-149</sup> A 1% increase in content of tissue water causes a decrease of x ray attenuation by 2 to 3 Hounsfield units (HU).<sup>150</sup> After experimental MCA occlusion, tissue water uptake is detectable by CT which shows a linear decline in x ray attenuation by about 1.5 HU/h.<sup>151</sup> Because of the normal noise in each CT image, a change of about 5 HU is necessary for an increase in tissue water to be visible.

Shrinkage of the extracellular space causes a decrease in proton diffusion which is best detected by diffusion weighted MRI.<sup>150-152</sup> The detection of ischaemic changes by diffusion weighted MRI is immediate.<sup>153</sup> The hyperintense area on the MRI representing diminished proton diffusion may be reversible during the first 2 hours after arterial occlusion, but later may correspond to irreversible tissue damage.<sup>154</sup> Therefore, the presence of visible subtle hypoattenuation on CT is consistent with a minimum 2% focal uptake of water and an arterial occlusion which occurred some time earlier. The degree of hypoattenuation

may be confounded by an increase in local cerebral blood volume, a well known compensatory mechanism for low perfusion pressure.<sup>155</sup>

From clinical experience, cerebral tissue hypoattenuation is regularly visible within the first 6 hours after the onset of ischaemic symptoms. The loss of the permeability barriers and entry of intravascular fluid, together with nonvascular cell injury contribute to early signs of ischaemic injury notable by CT: a regional decrease in  $x$  ray attenuation and evidence of structural changes, marked by mass effect. This hypoattenuation can at an early stage delineate a volume of parenchymal tissue that will subsequently become necrotic and, if exceeding 50% of the MCA territory, is associated with a mortality of 85%.<sup>156 157</sup> It seems likely, but remains unestablished, that even very subtle hypoattenuation on CT and areas with restricted diffusion detected by MRI indicate oedema and irreversible tissue damage caused by sustained focal hypoperfusion. Whether oedema formation and microvascular haemorrhage are two manifestations of the same microvascular injury processes is of central relevance.

#### Vascular contributors to ischaemic damage

The degree of ischaemic injury in a cerebral arterial territory also depends on the intrinsic vulnerability of the tissue within that region. The volume of ischaemic cerebral tissue may depend on (1) protection by the collateral circulation (regional vascular flow characteristics), and (2) the vulnerability of selected non-vascular cells (selective cellular vulnerability) to the vascular territory at risk.

Experimental studies and clinical experience suggest that the corpus striatum is more sensitive to ischaemic injury than the overlying cortex following proximal MCA occlusion.<sup>158-160</sup> This differential sensitivity is partly explained by vascular anatomical conditions: The cortex is protected by a net of leptomeningeal arteries which communicate with the parenchymal arterioles and between cortical territories, whereas the circle of Willis offers a continuous shunt of the basal brain supplying arteries.<sup>161</sup> Furthermore, the microvascular network of the cortex is distinctly different from that of the corpus striatum. Developmental features of the rat cortical microvasculature, described by Bär *et al*, indicate a roughly three dimensional hexagonal network extending from the pial vessels to the grey matter-white matter border.<sup>162 163</sup> This arrangement provides regional collateral vascular circuitry to shunt blood locally.<sup>164</sup> In the non-human primate and in humans, flow is directed from the MCA to the corpus striatum through lenticulostriatal arterial (LSA) perforators. Here, erythrocyte transit time is increased,<sup>165</sup> so that the regional CBF is closer to the threshold for ischaemic injury. In human patients and primates, the corpus striatum is particularly subject to unrecoverable injury after obstruction of the proximal (M1) MCA.<sup>159</sup> Occlusion of the proximal MCA causes an immediate drop in blood flow in the territories supplied by the LSA, whereas the superficial cortex may be salvaged by leptomeningeal collaterals.<sup>166</sup> In non-human primates after proximal MCA occlusion, haemorrhagic transformation is most often seen in the corpus striatum, where ischaemic injury is greatest.<sup>109</sup>

Selective vulnerability of neurons and glial cells is readily evident during experimental global cerebral ischaemia.<sup>167-171</sup> The Purkinje cells of the cerebellum, medium sized striatal neurons, and the CA1 pyramidal cells of the hippocampus are especially sensitive.<sup>172</sup> Histological damage may be apparent after about 3 minutes of ischaemia for hippocampal neurons, but after about 30 minutes for striatal neurons.<sup>172 173</sup> Furthermore, significant species and model differences in neuron vulnerabilities are possible.<sup>174 175</sup> Despite these distinct cellular

vulnerabilities, there is no evidence that during focal cerebral ischaemia they contribute to haemorrhage. Rather, this seems to be more dependent on the sensitivity of microvascular structures to ischaemia.

#### Haemorrhagic transformation in embolic stroke

Clinically, haemorrhagic transformation is manifest either by haemorrhagic infarction or parenchymal haematoma, or both.<sup>34 176</sup> Okada *et al*<sup>32</sup> and others<sup>31 38</sup> have shown that haemorrhagic infarction is more common than parenchymal haematoma in patients with carotid territory embolic stroke. Parenchymal haematomas seem to accompany acute carotid territory embolism more often in the setting of anticoagulant treatment.<sup>176-181</sup> The longstanding concept that haemorrhagic transformation may result from arterial reperfusion is so far not supported by angiographic studies.<sup>11 12 35 182</sup> Ogata *et al* have suggested that haemorrhage may also occur from collateral channels or other vascular sources.<sup>183</sup> Based on these clinical relations, it may be postulated that arterial reperfusion is dangerous when the microvascular basal lamina and matrix are disrupted by processes initiated by acute closure of the feeding artery. Regions of haemorrhagic transformation are likely to be those in which the microvascular beds are most vulnerable. Processes which disrupt microvascular structure (for example, amyloid deposition) may increase this vulnerability.

#### Plasminogen activators, ischaemic injury, and haemorrhagic transformation

Plasminogen activation carries an inherent risk of haemorrhage by altering platelet plug framework, vascular permeability, and vascular basal lamina integrity at sites of injury.<sup>184-186</sup> Exogenous PAs might accelerate dissolution of the blood-brain barrier, microvascular basal lamina/ECM, and platelet-fibrin plugs thereby increasing oedema formation and the risk of haemorrhage. This hypothesis is consistent with the finding that the frequency of haemorrhagic transformation in the setting of rt-PA exposure increases with the time of intervention from symptom onset in patients with MCA occlusion,<sup>35</sup> in whom intrinsically basal lamina/ECM dissolution increases with time from MCA occlusion.<sup>46</sup>

Five recent placebo controlled studies of intravenous PA infusion in acute ischaemic stroke highlight patient and study features which relate to the accentuated risk of haemorrhagic transformation (table 1). Three separate projects examined the relative benefits of  $1.5 \times 10^6$  IU streptokinase, the recommended dose for acute myocardial infarction.<sup>187 188</sup> Hommel and colleagues of the Multicenter Acute Stroke Trial-Europe (MAST-E) reported a significantly higher incidence of symptomatic intracranial haemorrhage in the streptokinase group compared with placebo among patients treated within 6 hours of the onset of an MCA territory stroke.<sup>5 9</sup> The significantly higher short term (10 day) mortality in the placebo group (18.1%), suggested that the increased risk of symptomatic parenchymal haemorrhage with streptokinase was due to the increased severity of strokes entered into that trial. The Australia Streptokinase (ASK) Trial, which randomised patients to intravenous placebo or streptokinase administered within 4 hours of acute ischaemic stroke, noted a significantly increased mortality among patients treated after 3 hours.<sup>4 6</sup> The time from symptom onset to treatment did not seem to be relevant to the excess frequency of symptomatic haemorrhage in the streptokinase group. A third study, the Multicentre Acute Stroke Trial-Italy (MAST-I), which compared intravenous streptokinase  $\pm$  aspirin and placebo within 6 hours of onset of symptoms, was terminated when interim analysis showed a significant

Table 2 Outcomes in patients (n (%)) with no or small hypoattenuation v large hypoattenuation on initial CT

Outcome event	Time (days)	CT findings within 6 h of stroke					
		Hypoattenuation ≤33% MCA territory			Hypoattenuation >33% MCA territory		
		Placebo	rt-PA	OR (95) CI	Placebo	rt-PA	OR (95) CI
PH	1	14/278 (5)*	47/269 (17)*	3.99 (2.14–7.44)	0/21 (0)*	9/31 (29)*	—
Mortality	7	20/279 (7)	25/272 (9)	1.31 (0.71–2.42)	6/21 (29)	9/31 (29)	1.02 (0.30–3.48)
Mortality	90	41/279 (15)	51/272 (19)	1.34 (0.85–1.34)	6/21 (29)	15/31 (48)	2.34 (0.72–7.63)
Rankin = 0+1	90	81/277 (29)*	108/265 (41)*	1.66 (1.17–2.38)	3/21 (14)	2/31 (6)	0.41 (0.06–2.72)

\*2p<0.01, Fisher's exact test between pairs within same hypoattenuation class.

PH=parenchymal haematoma. These data from the ECASS show for patients with an ischaemic oedema >33% of the MCA territory (as detected as hypoattenuation on the initial CT scan) an increase in PH, a significantly higher early and late mortality, and a significantly smaller proportion of patients with beneficial outcome in the rt-PA group. The 52 patients with large ischaemic lesions were identified by an independent CT scan evaluation. Small differences in the incidence of outcome events are due to missing values. — = Not calculable.

excess of 10 day case fatalities associated with streptokinase ± aspirin, but most particularly when streptokinase was given with aspirin.<sup>7,8</sup> The incidences of haemorrhagic transformation detectable by CT at 5 days and symptomatic cerebral haemorrhage during the hospital stay among patients treated with streptokinase ± aspirin significantly exceeded those in patients who received placebo.

ECASS, which tested the efficacy of rt-PA (alteplase) in acute ischaemic stroke applied within 6 hours of onset of symptom, did not show overall benefit in terms of disability outcome at 90 days.<sup>1</sup> In that study, symptomatic brain haemorrhage was not defined as a primary outcome event. Intracranial haemorrhage was assessed on days 1 and 7 after treatment and categorised as haemorrhagic infarction or parenchymal haematoma.<sup>176</sup> Haemorrhagic infarction on day 1 did not affect mortality outcome, which was 12% in patients with haemorrhagic infarction and 15% in patients without haemorrhage. However, parenchymal haematoma on day 1 was associated with a 52% mortality (von Kummer *et al*, unpublished data). The severity of initial clinical deficit and the presence of ischaemic changes on CT were associated with risk of haemorrhagic infarction. Increasing age and treatment with rt-PA were related to the risk of parenchymal haematoma.<sup>37</sup> In the rt-PA treated group, the risk of parenchymal haematoma increased with the extent of hypoattenuation on the initial CT.<sup>189</sup> In ECASS, the risk of cerebral haemorrhage was not apparently associated with the time from symptom onset to initiation of treatment, although the stroke population was heterogeneous with respect to aetiology.

The NINDS sponsored placebo controlled evaluation of rt-PA (alteplase) in patients with ischaemic stroke within 3 hours of onset of symptoms showed an 11% to 13% absolute improvement in best outcome using a composite of neurological outcome (NIHSS), two disability indices, and the Glasgow outcome score.<sup>2</sup> Within an apparent equivocal effect on mortality was a significant increase in symptomatic intracerebral haemorrhage from 0.6% in the placebo group to 6.4% in the rt-PA group. One interpretation of those data is that the improvement in clinical outcome would have been substantially increased had the frequency of symptomatic haemorrhagic transformation been attenuated. This interpretation also applies to the ECASS experience.<sup>1</sup>

The excessive frequency of early symptomatic intracerebral haemorrhage in each trial could be attributed to the use of a plasminogen activator, and in at least two trials also to the severity of injury.<sup>1,5</sup> In one trial, the impact of the injury on apparent risk may have been accentuated by an “excessive” dose of the streptokinase, and exposure of the injured microvascular bed to increased plasmin levels.<sup>5</sup> In a subgroup of patients with evidence of ischaemic injury on the initial CT scan entered into ECASS, exposure to rt-PA significantly increased the frequency of symptomatic parenchymal haematoma within 24 hours (table 2). That

experience implies that symptomatic haemorrhage complicates those regions with extensive early injury. Furthermore, a retrospective analysis of the NINDS dataset indicated that patients with severe strokes, or oedema, or mass effect on baseline CT had a higher frequency of intracerebral haemorrhage.<sup>3</sup> A similar finding has been made in a placebo controlled study of intra-arterial infusion single chain urokinase plasminogen activator.<sup>25</sup> These experiences are consistent with the postulate that the risk of symptomatic haemorrhage is a product of the depth and duration of ischaemic injury.

If it is true that the volume of brain tissue with irreversible ischaemic damage of microvessels is a dominant contributor to postschaemic cerebral haemorrhage, CT and MRI may identify patients with a potential increased risk of haemorrhage and mortality by exposure to PAs. Ueda *et al* have suggested that a threshold of rCBF reduction may be defined which is significantly associated with symptomatic intracerebral haemorrhage after PA exposure in acute stroke.<sup>190</sup> From ECASS, evidence of tissue injury exceeding 33% of the MCA territory was associated with increased early symptomatic haemorrhage and mortality.<sup>189</sup> The difficulty that CT and MRI findings during the first 2 hours may not be reliable because the disturbance of diffusion can be reversible<sup>191</sup> or the CT may not display a lesion although oedema formation is ongoing temper this impression. However, if CT shows hypoattenuation during the first 2 hours after onset of symptoms, a territory with severe ischaemia and oedema is identified. In ECASS, 60 of 152 (39%) patients examined within the first two hours of symptom onset showed hypoattenuation on their CT (which exceeded 33% of the MCA territory in 14 patients (9%)).<sup>189</sup> All patients with hypoattenuation on the initial CT independent of time of presentation showed necrosis on the follow up CT by 7 days (von Kummer, unpublished data).

Despite the apparent salutary effects of rt-PA in patients with ischaemic stroke treated within 3 hours of onset of symptoms,<sup>1,2</sup> studies selecting and treating patients with ischaemic stroke with PAs based only on clinical criteria have shown an excess of symptomatic haemorrhage.<sup>1,2,4-8</sup> This impression is supported by a recent meta-analysis.<sup>192,193</sup> Among smaller studies in which patients were selected on the basis of location of occlusion, with recanalisation as an outcome measure, the frequency of symptomatic haemorrhage did not differ significantly from those receiving placebo.<sup>194,195</sup> In either case, if the hypothesis is correct that PAs may augment the dissolution of the microvascular basal lamina/ECM and thereby exacerbate haemorrhage based on their common characteristic to generate plasmin, then cerebral haemorrhage is likely to be a consequence. But the generation of plasmin necessary for thrombus lysis is intrinsic to PA activity and the consequences of plasmin generation on microvascular integrity are so far unalterable. So, attempts to decrease the clinical

risk of plasminogen activator associated haemorrhagic transformation are necessary to improve overall outcome for patients and clinical studies.

The physician is currently left with applying strict criteria to select those patients with acute ischaemic stroke with attributes which may lower their intrinsic risk of haemorrhagic transformation. Specific characteristics which distinguish patients at risk for haemorrhage who receive a PA have not been resolved by experimental work and this is an important issue to be studied. Consideration of the roles that ischaemic microvascular injury may have in the processes of oedema formation and haemorrhagic transformation may guide that selection.

#### DEFINITION OF THE REGION OF ISCHAEMIC INJURY BY CT OR MRI

To reduce the inherent risk of intracerebral haemorrhage due to the PA, patients should be examined by CT or MRI to rule out primary haemorrhage as a cause of symptoms and to determine the volume of tissue which has had ischaemic injury. This is possible 2 hours after ictus, and in some patients even earlier. The critical volume of damaged tissue has yet to be determined. So far, a volume exceeding 33% of the MCA territory is associated with no benefit from PA and an increased risk for symptomatic haemorrhage.

#### DURATION OF INJURY

The designated time of onset of symptoms may underestimate the duration of ischaemic injury in some patients. Several uncontrolled and controlled studies have suggested that a decreased risk of haemorrhage is associated with shortened durations of ischaemic injury. Therefore, very early acquisition of patients to CT and potential treatment is required. Besides the need to initiate treatment very early, functional imaging methods to define the individual risk or extent of patients with sustained low rCBF on irreversibly damaged ischaemic tissue bear evaluation.

#### POTENTIAL CONTRIBUTORS TO MICROVASCULAR INJURY

The microvasculature is also a target for ischaemic and inflammatory injuries with the general consequences of increased permeability to plasma and circulating cells. Sustained exposure to raised blood pressure, diabetes mellitus, amyloid angiopathy, and comorbid inflammatory conditions (for example, vasculitis) are likely to augment the effects of ischaemia on the microvascular bed.

#### PLASMINOGEN ACTIVATORS AND THE MICROVASCULATURE

There is as yet no specific experimental data on the relative effects of various PAs on microvascular integrity. Whether certain PAs may promote additional injury is unknown. However, agents which initiate complement activation (for example, streptokinase)<sup>196-197</sup> and affect endothelial cell integrity (for example, streptokinase and rt-PA),<sup>184-198</sup> could augment ischaemic microvascular injury. This argues that specific dose adjustment studies and prudent choice of agents should be an integral part of the testing of PAs in this setting. Furthermore, events which augment basal lamina and ECM dissolution (for example, increased inflammation) are likely to increase the risk of microvascular haemorrhage.

These issues are appropriate for further well conceived experimental work, but must also rigorously apply to the approved use of plasminogen activators in acute ischaemic stroke.

#### Conclusions

(1) Present experimental data allow the hypothesis that loss of microvascular integrity is one prerequisite for the devel-

opment of haemorrhagic complications in focal cerebral ischaemia, with or without reperfusion.

(2) Loss of microvascular integrity may be increased by plasminogen activators although this has not been rigorously or prospectively examined.

(3) Because there is no currently available strategy to reduce or prevent loss of microvascular integrity during focal cerebral ischaemia, and therefore the intracerebral haemorrhagic complications, very careful patient selection based on clinical examination, CT, and perhaps MRI, must remain the current strategy.

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## NEUROLOGICAL STAMP

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### Ferdinand Jakob Heinrich von Müller (1825–96)

Ferdinand Jakob Heinrich von Müller was born in Rostock, Germany. Müller was a qualified pharmacist who then studied medicine but abandoned this due to his health. In 1847 he emigrated to Australia where he collected plants for research. He wrote an 11 volume book *Plants in Australia (1855–1881)*.

Many of Australia's native plants were sent overseas by Müller to chemists so that their curative properties might be investigated. He wrote and lectured on medical and poisonous plants, stressed the commercial importance of eucalyptus oil, and wrote extensively on *Cinchona calisaya*, from which quinine is prepared. He was appointed Director of the Melbourne Botanical Gardens and became a medical examiner for the University of Sydney. He received 184 civil and scientific honours during his life. For his contribution to biological science he was awarded the degree of Doctor of Medicine Honoris Causa in 1857 by the University of Rostock.

Philatelically Australia honoured him on a stamp issued in 1948. His portrait is shown with a sprig of the gum that bears his name, *Eucalyptus muelleriana*. (Stanley Gibbons 226, Scott 214).

L F HAAS

