Free Radical Research, April 2009; 43(4): 348–364

informa
healthcare

Involvement of ROS in BBB dysfunction

PAMELA B. L. PUN, JIA LU, & SHABBIR MOOCHHALA

Defence Medical and Environmental Research Institute, DSO National Laboratories, Singapore

(Received 29 November 2008; revised 2 January 2009)

Abstract
The blood–brain barrier (BBB) forms a protective barrier around the brain, with the important function of maintaining brain homeostasis. Pathways thought to initiate BBB dysfunction include the kinin system, excitotoxicity, neutrophil recruitment, mitochondrial alterations and macrophage/microglial activation, all of which converge on the same point—reactive oxygen species (ROS). Interestingly, ROS also provide a common trigger for many downstream pathways that directly mediate BBB compromise such as oxidative damage, tight junction (TJ) modification and matrix metalloproteinases (MMP) activation. These observations suggest that ROS are key mediators of BBB breakdown and implicate antioxidants as potential neuroprotectants in conditions like stroke and traumatic brain injury (TBI). This review explores some of the pathways both upstream and downstream of ROS that have been implicated in increased BBB permeability and discusses the role of ROS and antioxidants in neuropathology.

Keywords: *Reactive oxygen species, blood–brain barrier, oxidative stress, antioxidants, neuropathology*

Abbreviations: *3-NP, 3-nitropropionic acid; 4-HNE, 4-hydroxynonenal; AA, arachidonic acid; ABAD, amyloid beta-binding alcohol dehydrogenase; AD, Alzheimer's disease; ALCAM, activated leukocyte cell adhesion molecule; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; ARE, antioxidant response element; Aβ, beta-amyloid; BBB, blood–brain barrier; BMVECs, brain microvascular endothelial cells; cAMP, cyclic adenosine monophosphate; cDNA, complementary DNA; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; COX, cyclooxygenase; DAG, diacylglycerol; eNOS, endothelial NOS; EPO, erythropoietin; ERK, extracellular signal-regulated kinase; F-actin, filamentous actin; FAK, focal adhesion kinase; GSH, glutathione; H2O2, hydrogen peroxide; ICAM, intercellular adhesion molecule; IFNγ, interferon-gamma; IL, interleukin; iNOS, inducible NOS; IP3, inositol triphosphate; JAK, Janus kinases; Keap1, Kelch-like ECH-associated protein 1; MAP, mitogen-activated protein; mDIA, mammalian diaphanous-related forming; MLC, myosin light chain; MLCK, MLC kinase; MMP, matrix metalloproteinase; MS, multiple sclerosis; Na+/K+-ATPase, sodium-potassium ATPase; NADPH, nicotinamide adenosine dinucleotide phosphate; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; Nrf-2, nuclear factor E2-related factor 2; ONOO−, peroxynitrite; PECAM, platelet endothelial cell adhesion molecule; PI3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLA2, phospholipase A2; PLC, phospholipase C; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; RhoGEF, Rho guanine-nucleotide exchange factor; ROCK, Rho kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; STAT, signal transducers and activators of transcription; TBI, traumatic brain injury; TEER, transendothelial electrical resistance; TJ, tight junction; TNFα, tumour necrosis factor-alpha; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; ZO, zonulae occludens*

Correspondence: Shabbir Moochhala, Combat Care Laboratory, Defence Medical and Environmental Research Institute, 27 Medical Drive, Singapore 117510. Fax: +65 6485 7226. Email: mshabbir@dso.org.sg

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760902751902

Introduction

The blood–brain barrier (BBB) is a protective membranous barrier that restricts the entry of molecules and white blood cells from the systemic circulation into the central nervous system (CNS). It thus functions to maintain the homeostatic balance of the brain extracellular fluid, thereby ensuring normal brain function.

The BBB consists of the capillary basement membranes, brain microvascular endothelial cells (BMVECs), astrocytic endfeet and pericytes. The astrocytic endfeet function in brain water homeostasis, which together with the pericytes, have been implicated in BBB development and permeability, although their precise role in the BBB remains in dispute [1,2]. The major component of the BBB is the BMVECs. Unlike endothelial cells in the peripheral circulation, BMVECs lack fenestrations, thus preventing the passage of molecules across the BBB through these gaps. In addition, BMVECs have lower pinocytic activity than other endothelial cells [3], thus minimizing transcellular transport of substances across the BBB. Tight junctions (TJs) and adherens junctions further contribute to the barrier property by limiting paracellular diffusion. While the latter consists of cadherin and catenin, the former contains occludin and claudins. These are transmembrane proteins whose expressions are closely associated with BBB permeability. For instance, increased occludin expression decreases paracellular transport and thus reduces permeability across the BBB [4], while claudin-5 prevents the passage of large molecules [5]. Occludin and claudins are linked to the actin cytoskeleton via cytoplasmic proteins from the zonulae occludens (ZO) family. Together with signalling molecules such as Rho, PI3K and calcium ions (Ca^{2+}), these components regulate TJ integrity and BBB permeability.

BBB integrity is compromised when the flux of molecules through either the paracellular or the transcellular pathway is increased. This could happen if pinocytic activity increases or if TJs open. BBB breakdown has been reported in various neurological conditions such as traumatic brain injury (TBI), multiple sclerosis (MS), stroke and stress [6–9]. Interestingly, a common feature in all these conditions is oxidative stress, a situation in which the oxidant–antioxidant balance is disturbed, resulting in excess oxidants. Reactive oxygen species (ROS) levels are known to increase in such pathology and it has been postulated that these ROS contribute to increased BBB permeability. In fact, experiments in frogs have shown a correlation between increased ROS levels and decreased electrical resistance across the brain endothelium, indicating an increase in BBB permeability [10]. In addition, disturbances in antioxidant levels and oxidative damage have been associated with BBB dysfunction. For instance, depletion of cerebral glutathione (GSH) in rat brains led to increased BBB permeability, while restoration of normal GSH levels returned permeability back to normal [11]. Because GSH is an important physiological antioxidant, such observations further support the idea that oxidative stress is a crucial determinant of BBB permeability. Several issues pertaining to this hypothesis are discussed in this review.

How do ROS influence BBB integrity?

The initial insult in neuropathologies may vary from ischemia in stroke to environmental toxins in Parkinson's disease. Regardless of the initial insult, the commonality in these conditions is increased ROS production. Several pathways may link the initial insult to the rise in ROS production, some of which are discussed below.

How do such damaging ROS levels arise?

Bradykinin system. Bradykinin is formed from kinogen in a process catalysed by kallikrein. It is one of the first agents released upon inflammation and injury and has been found to increase in plasma and brain tissue following cerebral ischemia/reperfusion [12]. This, coupled with observations that kinin antagonists can reduce oedema following strokes in animals [13] while kinin receptor agonists can increase BBB permeability [14], indicates the involvement of bradykinin in BBB breakdown. There are several mechanisms by which bradykinin, upon binding to B2-receptors in the CNS, can increase BBB permeability.

For one, bradykinin can activate phospholipase A_2 (PLA_2), which then cleaves membrane phospholipids to release arachidonic acid (AA) [15]. It has been postulated that ROS produced during AA metabolism directly modulates BBB integrity. For instance, inhibition of 5-lipoxygenase (which converts AA to leukotrienes) reduced AA-induced oedema [16]. It has also been shown that bradykinin-induced BBB permeability occurs via ROS produced during cyclooxygenase (COX) metabolism of AA [17] and that COX inhibition prevented BBB breakdown [18]. COX inhibition in conjunction with inducible nitric oxide synthase (iNOS) inhibition also improved cerebral tissue perfusion in rats following TBI [19]. It thus appears that inhibition of AA metabolism and, in so doing, decrease in ROS production, protects BBB integrity and improves post-injury prognosis. However, it is also possible that it is the metabolites of AA, namely leukotrienes and prostaglandins, rather than the ROS by-products, which increase BBB permeability, a possibility discussed previously [20,21]. Nonetheless, the possible involvement of

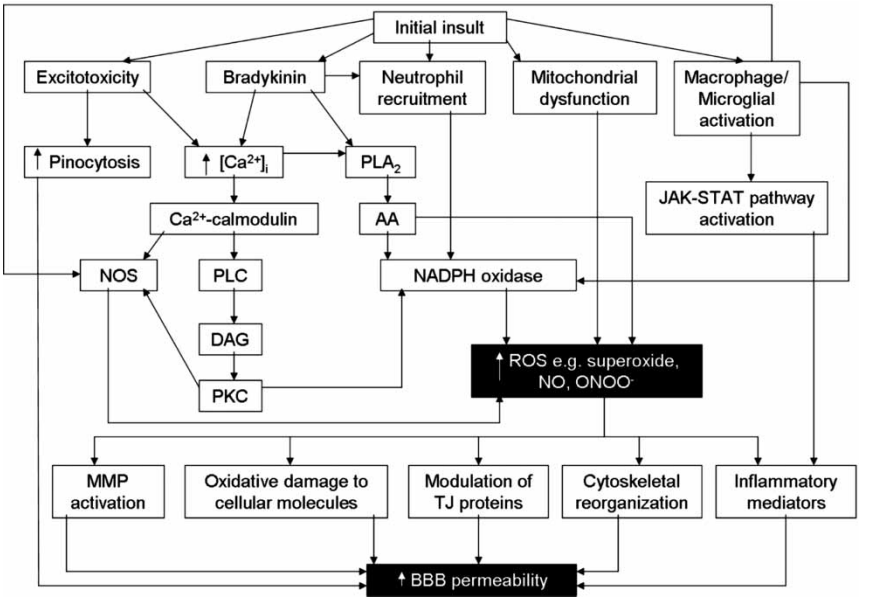

Figure 1. Pathways by which an initial insult can increase ROS generation and by which ROS can cause BBB breakdown.

ROS cannot be dismissed, especially when it appears to be a converging point across various mechanisms of BBB breakdown, as will be discussed below and as depicted in Figure 1. Besides, AA may also facilitate NADPH oxidase activity, thus increasing ROS production further [22].

Apart from increasing AA production from membrane phospholipids, bradykinin can also induce BBB opening by increasing Ca^{2+} levels within endothelial cells. In fact, an increase in intracellular Ca^{2+} levels has been correlated with a decrease in transendothelial electrical resistance (TEER), while antagonizing the key bradykinin receptor B2 prevented this Ca^{2+} spike and drop in TEER [16]. Interestingly, glutamate-receptor antagonists also prevented this bradykinin-related rise in Ca^{2+} levels [23], suggesting a link between excitotoxicity (commonly associated with glutamate) and bradykinin-induced BBB breakdown.

There are several ways by which Ca^{2+} elevation can stimulate ROS production. For example, Ca^{2+}, via the Ca^{2+}-calmodulin complex, can activate nitric oxide synthase (NOS) [24]. NOS appears particularly important to bradykinin-induced BBB opening as cells expressing low levels of NOS were resistant to the effects of bradykinin, a sharp contrast against those which had high levels of NOS expression [25]. The exact NOS isoform involved remains a subject of debate which we discuss later in this review. NOS activation leads to increased nitric oxide (NO) production [24]. NO and the NO-derived peroxynitrite ($ONOO^-$) may then increase BBB permeability by affecting TJ proteins [26]. NO could also induce BBB breakdown by activating the cGMP-PKG cascade. This cascade has been shown to decrease BBB resistance *in vivo* and in intact perfused microvessels [27,28]. Thus, NO could elevate BBB permeability by modification of TJ proteins or via the cGMP-PKG pathway.

The second pathway which Ca^{2+} can activate is the PLC–DAG–PKC pathway. Ca^{2+} activates PLC, which cleaves PIP_3 to produce IP_3 and DAG [29]. DAG in turn activates PKC which causes BBB disruption either by modulating the actin cytoskeleton or by increasing NO production via NOS [30]. Alternatively, PKC activation could also lead to the up-regulation and activation of NADPH oxidase, leading to increased ROS production [31,32].

Thirdly, Ca^{2+} can activate PLA_2, stimulating AA production [33]. AA, as earlier described, can in turn cause an increase in ROS production.

In summary, bradykinin can cause BBB breakdown either by increasing AA production or by increasing intracellular Ca^{2+} levels, both of which ultimately lead to elevated ROS generation. Because various compounds including apocynin (an NADPH oxidase inhibitor) [34], indomethacin (a non-selective COX inhibitor) and N-acetylcysteine (an antioxidant) [35] are known to inhibit different pathways associated with bradykinin and to ameliorate BBB dysfunction, it appears that the extent and severity of bradykinin-induced BBB opening may be determined by the cross-talk between the various pathways described above.

Excitotoxicity. Excitotoxicity is a situation in which there is an overload of excitatory amino acids like glutamate or excitotoxins, resulting in pathology via ways such as increased ROS production and elevated AA generation.

Glutamate is a central molecule in several neurological conditions [36–38]. For instance, in TBI,

glutamate release is among the first events to occur post-injury [6]. Binding of excess glutamate and/or excitotoxins to NMDA/AMPA receptors causes increased Ca^{2+} entry via these receptors. Influx of sodium ions (Na^{+}) via the AMPA receptors also stimulates Ca^{2+} entry by reversing the Na^{+}/Ca^{2+} transporters. Binding of glutamate or excitotoxins to metabotropic glutamate receptors further increases intracellular Ca^{2+} levels by stimulating the release of intracellular Ca^{2+} stores from the endoplasmic reticulum. It is interesting to note that, although the pathway by which glutamate causes BBB disruption resembles that by which it causes neuronal death, the two processes actually occur independent of each other [39]. Thus, it appears that neuronal death is not a causative factor in BBB dysfunction.

Apart from inducing oxidative stress, excitotoxicity could also aggravate BBB disruption by destroying astrocyte function, thus preventing repair of the BBB [39]. BBB permeability could also be increased due to an elevation of pinocytic rate across endothelial cells following excitotoxicity [40].

Neutrophil recruitment. Upon injury, neutrophils are recruited to the BBB as part of the inflammatory response [41]. Neutrophils have been implicated in ischemic injury, with reports showing that hypoxia increases neutrophil 'lifespan' [42] and that an increase in superoxide generation by neutrophils occurs during ischemia [43]. In fact, BBB dysfunction has been shown to result from neutrophil recruitment [44]. Thus, neutrophils play an important role in BBB disruption.

Activated neutrophils are a major source of ROS during inflammation, with enzymes like NADPH oxidase catalysing the production of ROS [45]. Neutrophils express B2-receptors for bradykinin [46], and bradykinin, as earlier discussed, can lead to AA generation and PKC activation. Both these events have been shown to activate NADPH oxidase [47,48]. Therefore, it seems plausible that ROS production by neutrophils in neuropathology may be bradykinin-dependent. It has to be stressed however that this hypothesis has yet to be verified experimentally to our best knowledge. The ROS thus produced could then adversely affect BBB integrity via TJ proteins modification [49] or via the expression of inflammatory mediators, as will be discussed later.

Mitochondria. Mitochondria are the major sources of ROS production in most mammalian cells [50]. Such production increases in many pathological conditions and is associated with mitochondrial dysfunction [51–53]. Interestingly, although mitochondrial ROS production is largely attributed to complexes I and III [54], not much work has been done on these complexes in relation to BBB dysfunction to our best knowledge. Instead, of the electron transport chain complexes, complex II has assumed a more prominent role in this context as described below.

The mitochondrial toxin, 3-nitropropionic acid (3-NP), is commonly used to produce a model of Huntington's Disease in animals [55]. 3-NP adversely affects mitochondrial integrity and function in several ways. For instance, it has long been known to irreversibly inhibit complex II [56]. More recently, 3-NP was observed to cause significant complex I dysfunction *in vivo* [57]. Such findings suggest that 3-NP may increase ROS production in the mitochondria. After all, inhibition of both complexes I and II are known to lead to enhanced/increased superoxide generation [54,58,59]. The mitochondrial toxin also increases markers of oxidative damage and matrix metalloproteinase (MMP)-9 expression in injured striatum, concurrent to inducing BBB opening, with the severity of 3-NP-induced striatal damage being correlated to SOD expression [60]. Taken together, these findings suggest that 3-NP, by disrupting the mitochondrial electron transport chain, causes an elevation of ROS generation, thereby triggering events such as MMP-9 induction that eventually lead to BBB opening.

Macrophage infiltration and activation. Macrophage accumulation is commonly observed in the brain following injury [61,62]. It remains unclear if these macrophages originated from resident microglia already in the brain or if they were differentiated from blood-derived monocytes that had migrated across the BBB. Two groups have separately observed that, in the absence of blood-derived monocytes, brain microglia is able to differentiate into macrophages, with one group further observing that these macrophages can go on to activate the complement pathway, consequently causing BBB breakdown [63,64]. This suggests that macrophages that accumulate in the injured brain are derived from brain microglia. However, because it is difficult to distinguish between macrophages of the two possible origins, the contribution of blood-derived monocytes cannot be ruled out [61].

Regardless of their origin, macrophage/microglial activation seems to be an early event in injury [65] that precedes BBB breakdown [66], with inhibition of their activation preventing BBB dysfunction [67]. It appears though that macrophage accumulation occurs at a later time post-injury than neutrophil recruitment, suggesting that neutrophils may play a more important role in the acute phase [61]. However, we feel that the time course of leukocyte recruitment and activation may vary depending on the nature of the injury, especially since significant macrophage accumulation/activation is known to occur in the acute phase of ischemia, but only sets in 72 h post-TBI [68].

Although a major source of ROS generation, the influence of macrophage/microglial activation on the brain has already been extensively reviewed elsewhere [65,69] and shall not be covered in detail here. Suffice to say, activated macrophages/microglia produce ROS via NADPH oxidase [70–72]. Such ROS are not only cytotoxic [73] but can also activate redox signalling pathways such as the JAK-STAT pathways, thereby triggering an inflammatory response [74]. For instance, activated macrophages/microglia express C1q [64,75], which induces the expression of pro-inflammatory mediators such as TNFα and Egr-1, all of which can contribute to BBB disruption [66]. Activated macrophages/microglia may also express iNOS, thereby generating significant and possibly damaging levels of NO [76,77].

The pathways involved in BBB dysfunction upstream of ROS are summarized in Figure 1. It should be noted that the pathways described here and below are by no means exhaustive. Other pathways such as the cAMP-PKA pathway may also be involved in BBB breakdown [78]. These are, however, not discussed here as their role in ROS-induced BBB dysfunction remains unclear.

How does oxidative stress affect the BBB?

Oxidative damage to cellular molecules. An increase in oxidative stress can lead to elevated oxidative damage to biomolecules such as proteins and lipids. For instance, GSH depletion increases the susceptibility of protein sulphydryls to oxidative insult and has in fact been shown to cause membrane protein damage at the BBB and consequently increased BBB permeability [79]. In another example, decrease of Na^+/K^+-ATPase activity was closely correlated to the induction of oxidative stress, suggesting that elevated ROS levels cause damage to the ATPase, thereby leading to BBB dysfunction by allowing excessive and inappropriate influx of Ca^{2+} into cells [80].

Membrane lipids form another important constituent of the BBB, providing a large surface area across which lipid-soluble molecules can diffuse via the transcellular pathway. Membrane lipids could be oxidized to give cytotoxic lipid peroxidation products like malondialdehyde and 4-hydroxynonenal (4-HNE) which may adversely affect BBB integrity. For instance, the addition of exogenous 4-HNE increased the permeability of an *in vitro* BBB model [81]. Conversely, treatment of cells with inhibitors of lipid peroxidation products protected against BBB damage [82,83]. It thus appears that lipid peroxidation increases BBB permeability, probably by modulating the transcellular passage of substances.

ROS can also influence BBB integrity via its effects on DNA. For instance, elevated ROS levels causes hypermethylation of the promoter region of E-cadherin, resulting in E-cadherin down-regulation [84], corroborating with observations that endothelial cell expression of E-cadherin is decreased following hypoxia [85]. E-cadherin is important to BBB function [86]. Therefore, although a direct relationship between ROS-induced E-cadherin down-regulation and BBB leakiness has not been demonstrated, it is plausible that down-regulation of E-cadherin, presumably as a result of elevated ROS levels, causes BBB breakdown.

Changes in TJ proteins. As mentioned earlier, occludin, claudin and ZO proteins are components of TJs which regulate the paracellular pathway, thereby governing the passage of water-soluble molecules and ions across the BBB [87]. Any alterations in these proteins therefore will be expected to influence BBB permeability. Indeed, it has been found that exposure to a $ONOO^-$ donor induced a decrease in claudin-5 content [26]. Claudin-5 is a transmembrane protein that prevents molecules greater than 800 Da from passing through the BBB [5]. Thus, a decrease in claudin-5 content will be expected to increase BBB permeability to molecules larger than 800 Da.

Similarly, exposure of murine BMVECs to hypoxia followed by reoxygenation [88] and rat BMVECs to xanthine/xanthine oxidase [89] reduced occludin expression. Interestingly, another study using bovine BMVECs exposed to hydrogen peroxide (H_2O_2) found an increase in occludin content instead [90]. However, despite this rise in occludin expression, most occludin was dispersed throughout the cell membranes rather than being concentrated at the TJs. Hence, despite the disparate effects of ROS exposure on occludin expression *per se*, the outcome is consistent—there is increased BBB permeability and BBB dysfunction. The reason for the differences in effects on occludin expression is unclear, although it may be due to differences in the nature of ROS involved and the type of cells used.

Claudin and occludin aside, ROS could also alter BBB permeability by influencing ZO protein distribution. For instance, exposure to H_2O_2 led to a redistribution of ZO-1 from the TJs to the cytosol, resulting in a decrease in TEER and an increase in BBB permeability [90].

In addition to affecting the expression and distribution of TJ proteins, oxidative stress could also compromise barrier function by influencing the phosphorylation of these proteins. Alcohol-induced oxidative stress was found to increase serine phosphorylation on claudin-5 and occludin and serine/threonine phosphorylation on ZO-1 [26]. A switch in phosphorylation from serine to threonine residues on claudin-5 is associated with increased BBB resistance [91]. It is thus plausible that the reverse, that is an increase in serine phosphorylation, would decrease BBB resistance. A shift in phosphorylated residues on

occludin and ZO-1 could also influence TJ protein localization [26,92], thus playing a role in BBB breakdown.

In short, the expression, distribution and phosphorylation of TJ proteins are of utmost importance to BBB permeability. A change in any of these parameters induced by ROS could thus compromise BBB integrity. The signalling molecules involved in evoking such changes are uncertain, although candidates include the MAP kinase ERK [88], PI3K [89] and RhoGEFs [34].

Cytoskeletal reorganization. Elevated ROS levels may also alter BBB integrity by causing cytoskeletal changes. For instance, superoxide induces F-actin stress fibre formation in BMVECs within 30 min of exposure, possibly via a Rho-dependent pathway [89,93]. Upon activation, Rho phosphorylates various proteins such as FAK [94], ROCK and mDIA [95]. Activation of ROCK then leads to increased myosin light chain (MLC) phosphorylation both directly and indirectly via inhibition of MLC phosphatases [95]. Oxidative stress also leads to increased expression of chemokine receptors [96]. Increased signalling flux through these receptors further contributes to MLC phosphorylation via the activation of myosin light chain kinase (MLCK) [96], thereby modulating actin structure. MLCK can also phosphorylate TJ proteins, further disturbing the cytoskeletal organization. For instance, exposure to peroxide, $ONOO^-$ and NO increases serine phosphorylation on occludin [26], leading to changes in actin-cytoskeleton interactions [92] and hence increasing BBB permeability [97].

MMP activation. MMPs are zinc-containing proteolytic enzymes that degrade components of the extracellular matrix and of basement membranes. MMP inhibition prevents BBB opening [60], possibly by reducing occludin loss and preventing endothelial gap formation [98]. Therefore, it appears that MMP activity is a crucial determinant in BBB permeability. The precise molecular mechanism by which MMPs are activated is unclear. However it has been found that protein tyrosine kinase (PTK) inhibitors can prevent MMP activation [99], thus implying a role for PTKs. In addition, SOD2-knockout mice, which have decreased antioxidant capacities, are known to suffer greater BBB dysfunction involving MMP activity [100] while SOD1 over-expression reduces MMP-9 activation [101]. Both these pieces of evidence support the idea that the initial trigger for MMP activation involves ROS. Therefore it appears that, under conditions of oxidative stress, PTKs are activated [102]. PTKs in turn may lead to MMPs activation. Once activated, MMPs degrade the endothelium basement membrane, leading to increased BBB permeability [103].

Inflammatory mediators. The transcription factor NF-κB is activated in a redox-dependent manner. Upon activation by ROS, NF-κB can stimulate the expression of the adhesion molecules ICAM-1 and VCAM-1 [104]. ICAM-1 cross-linking can then activate Ca^{2+}-signalling pathways, leading to cytoskeletal alterations in BMVECs [105], thus causing BBB compromise. Adhesion molecules could also contribute to BBB opening by mediating leukocyte-vascular adhesion, inhibition of which prevents BBB dysfunction [106]. For instance, the induction of ICAM-1 and VCAM-1 on endothelial cells promotes the recruitment of activated neutrophils and leukocytes. Similar to ROS-induced cytoskeletal disorganization, such recruitment appears to be Rho-dependent [107]. The recruited cells then release inflammatory mediators like TNFα and IL-1β, leading eventually to BBB breakdown [44,108,109]. Furthermore, as these cells move across the BBB, they may also induce changes in the BBB structure. The movement of monocytes across the BBB, for example, causes loss of occludin at the TJs, thus increasing BBB permeability [98]. Recruited leukocytes such as neutrophils and macrophages may also exacerbate injury to the BBB by producing even more ROS. Besides ICAM-1 and VCAM-1, alpha integrins such as CD11b have also been implicated in leukocyte recruitment [110] and the expressions of these are also thought to be redox-dependent [111,112]. Likewise, other adhesion molecules such as PECAM-1 [113], E-selectin [114] and P-selectin [115] have also been reported to be redox-regulated. However, their influence on BBB permeability is either poorly characterized and largely hypothetical [116] or highly variable depending on the stimuli used [117]. Conversely, adhesion molecules such as ALCAM have been implicated in BBB dysfunction [118], but are not known to be influenced by ROS, and these are not discussed here.

Another commonly implicated inflammatory mediator is VEGF. It has been shown that exposure of osteoblasts and of human retinal epithelial cells to ROS causes an increase in VEGF expression [119,120]. It is plausible that the same happens in BMVECs, leading to BBB dysfunction. After all, VEGF has been shown to alter the distribution, expression and phosphorylation of TJ proteins like ZO-1 and occludin [121,122] and to increase the permeability of cultured BMVECs [123]. Therefore, it is likely that ROS induce VEGF expression in BMVECs, triggering a cascade of events that eventually leads to BBB breakdown.

The pathways involved in BBB dysfunction downstream of ROS are summarized in Figure 1.

Is ROS-induced BBB damage reversible?

Few works have been published that specifically evaluated the reversibility of the effects which ROS

have on the BBB. In one paper, it was reported that the increase in permeability to sucrose of rat brain endothelial cell monolayers induced by menadione (a redox-cycling agent) was reversed upon removal of menadione from the culture medium [124]. Another group found that the decrease in BBB resistance following trauma was in part due to a '*transient* expression of transendothelial vesicular transport system' [125], suggesting a lack of permanence of effect. Subsequently, a third group observed that in rats expressing a vector carrying IL-1β cDNA, there was an initial increase in BBB permeability, but this was reversed by day 30 post-injection [44]. All these findings suggest that ROS-induced BBB breakdown is reversible.

This is not totally unexpected, as many of the processes by which ROS are thought to cause BBB dysfunction are themselves reversible. For instance, MMPs may be activated by ROS via an increase in flux in the PTK pathway. This could occur either by activation of PTK and/or by inhibition of protein tyrosine phosphatases (PTPs), both of which are reversible processes [126]. Similarly, H_2O_2-induced cytoskeletal alterations in endothelial cell cultures can be reversed upon H_2O_2 removal by catalase [127]. Thus it is unlikely that ROS will cause a permanent dysfunction of the BBB, something that is generally supported by scientific literature. However, in chronic conditions where elevated ROS production is sustained, it is possible that the resulting damage is permanent. More work remains to be done to confirm this.

Which ROS are principally involved in BBB damage?

Despite the apparent importance of ROS in regulating BBB integrity, it remains unclear which species of ROS is principally involved. Several pieces of evidence point to a key role for superoxide:

(a) Inhibition of NADPH oxidase protects against BBB dysfunction [34] and NADPH oxidase produces superoxide.
(b) SOD catalyses the dismutation of superoxide and SOD over-expression attenuates BBB dysfunction [101], whereas SOD knockout aggravates BBB damage [100].
(c) Superoxide generated by the xanthine/hypoxanthine system induces stress fibre formation and cytoskeletal changes in rat brain endothelial cells and modulates changes in the BBB [93].
(d) Superoxide was definitively identified in a cat model of ischemic-reperfusion injury in which its generation was detected in the endothelium and vascular smooth muscle [128]. Treatment of the same model with SOD alone reduced the increase in BBB permeability [129].

Although the above results suggest that superoxide is the ultimate trigger in BBB dysfunction, they do not address the question of whether BBB damage is directly mediated by superoxide or by other species derived from superoxide, for example, H_2O_2. After all, exogenous H_2O_2 has been shown to increase BBB permeability both time- and dose-dependently [90]. Therefore, it is possible that superoxide is first dismutated to H_2O_2 which then directly exerts damaging effects on the BBB. This appears to contradict an earlier finding that pre-treatment of a cat model of ischemic-reperfusion injury with catalase alone failed to attenuate BBB dysfunction, whereas SOD was an effective prophylaxis [129]. Given how catalase removes H_2O_2 and how exogenous H_2O_2 has clearly been demonstrated to cause BBB leakiness, it is unclear why catalase should have been ineffective. We speculate that the apparent disparity may be a result of low *in vivo* H_2O_2 concentrations in the cat model of injury. Since the effect of H_2O_2 on BBB opening is dose-dependent, assuming that *in vivo* H_2O_2 concentrations are very low, it then follows that catalase would not significantly improve BBB dysfunction. It therefore appears that, relative to superoxide, H_2O_2 may not be an important mediator, if at all, of BBB breakdown *in vivo*. However, because no measurement of *in vivo* H_2O_2 concentrations in relation to BBB permeability has been made to our best knowledge, it would be inappropriate to make a definitive conclusion on this matter at this point in time.

Besides H_2O_2, another ROS derived from superoxide is $ONOO^-$. $ONOO^-$ is formed from superoxide and NO. $ONOO^-$ is a potent oxidant and has been shown to mediate BBB dysfunction [130].

Given the neurotoxicity of $ONOO^-$, it would be expected that NO, from which $ONOO^-$ is formed, should similarly be neurotoxic. Indeed, it has been shown that NO increases BBB permeability both *in vitro* [131] and *in vivo* [132]. However, one study found that NO prevents malondialdehyde formation and attenuates BBB breakdown induced by hypoxia-reperfusion injury in rat BMVECs [133]. Thus, the role of NO in BBB function is disputable. The apparent disparity in these studies may be related to the levels of NO involved. It may be that NO at low concentrations scavenges superoxide, preventing the harmful effects of superoxide on the BBB. Although $ONOO^-$ will be produced in the process, levels of $ONOO^-$ will be very low due to low NO concentrations and thus be insufficient to cause significant neurological damage. In contrast, at high NO concentrations, large amounts of $ONOO^-$ are produced and this results in BBB dysfunction. However it has to be emphasized that this is by no means a proven truth as no experiments to our best knowledge have been conducted to verify this.

Just as the effects of NO remain in dispute, it is also unclear which NOS isoform is primarily involved in BBB breakdown. While iNOS is generally believed to account for the high level of NO generated during inflammation [134] and inflammation in turn is associated with ROS production and BBB dysfunction, more recent reports appear to suggest that iNOS is not involved in NO-mediated BBB breakdown. For instance, while the endothelial NOS (eNOS)-specific inhibitor L-NIO decreased glutamate-induced NO production in murine brain endothelial cells, the iNOS-specific inhibitor 1400W had no effect [135]. A separate study found no correlation between the expression of iNOS and changes in BBB integrity induced by IFNγ [136]. Besides, it has been shown that the Ca^{2+}-chelator BAPTA prevents ROS generation and BBB breakdown [137], indicating that ROS-induced BBB dysfunction is Ca^{2+}-dependent. However, iNOS activity is independent of Ca^{2+} [138], suggesting that iNOS may not be involved in ROS-mediated BBB breakdown. In addition, as discussed earlier, loss of BBB integrity as mediated by ROS is generally reversible. However, iNOS-mediated NO production is long-lasting, a stark contrast to the short-lived increase in NO generated by eNOS. These observations collectively suggest that eNOS, rather than iNOS, is the principal mediator of NO production involved in BBB breakdown. However, in a mouse model of meningitis, iNOS, together with eNOS, was found to be expressed at higher levels than in healthy controls [139]. Rat models of closed head injury also displayed increased expression of iNOS, although eNOS was also observed to be up-regulated [140]. Furthermore, inhibition of iNOS by its specific inhibitor aminoguanidine prevented BBB dysfunction in rats [141]. Thus, it remains to be seen if iNOS is indeed dispensable to ROS-mediated BBB disruption. It may be that different NOS isoforms are involved in different cells or neuropathology. More work has to be done before a clearer picture emerges.

Therefore, while superoxide, H_2O_2, $ONOO^-$ and NO have all been implicated in BBB dysfunction, it is uncertain what their precise roles and importance are. This is not surprising as free radicals at low levels can be useful species with physiological roles, yet, at higher concentrations, be highly damaging oxidants. More studies to determine how different doses of these ROS affect BBB function and integrity are necessary before definite conclusions can be drawn.

What is the relevance of ROS in neuropathology?

ROS are produced in the early stages soon after neurological damage [100]. As discussed above, these ROS can then go on to activate enzymes and cause oxidative damage, resulting in BBB dysfunction. ROS have been implicated in several neurological conditions such as Alzheimer's disease (AD) [142], stroke [143] and MS [144]. As it is impossible to discuss all neuropathology, only a few better studied examples will be briefly described below.

AD is a neurodegenerative condition typically characterized by beta-amyloid (Aβ) deposits and neurofibrillary tangles [142], with increased BBB permeability relative to age-matched controls having been documented in human AD patients [145,146]. It appears that BBB breakdown is an early event in AD [147] and the severity of BBB opening could influence disease progression in AD patients [148].

BBB opening in AD has been attributed to small soluble aggregates of Aβ [149], which are also known to increase ROS production in neuronal and microglial cell cultures [150,151]. Observations that protein and DNA oxidative damage are increased in AD brains [152,153] further support the idea of increased oxidative stress in AD. It appears that there are several ways by which Aβ elevates ROS production:

(a) Aβ complexes to copper ion (Cu^{2+}) which can be reduced in a reaction that is coupled to the oxidation of molecular oxygen to H_2O_2, thus raising H_2O_2 levels. The Aβ radical generated in the process can also cause oxidative damage by reacting with cellular macromolecules, thereby producing protein carbonyls and lipid peroxidation products [154].
(b) Intra-mitochondrial Aβ can bind to the mitochondrial enzyme, amyloid beta-binding alcohol dehydrogenase (ABAD), forming a complex which inhibits complexes III and IV of the mitochondrial electron transport chain [155], consequently increasing ROS formation [156].
(c) Aβ activates signalling flux through NMDA receptors, thereby causing an increase in intracellular Ca^{2+} [157]. This Ca^{2+} flux activates PLA_2 and NADPH oxidase (Figure 1), resulting in elevated ROS generation [151,158].

The precise link between Aβ-induced oxidative stress and BBB opening is unclear. However, decrease in occludin expression, re-distribution of claudin-5 and ZO-2 from the membrane to the cytoplasm [159] and increased MMP expression [160,161] have all been documented in both human and animal models of AD. As described earlier in this review, these changes are all known to be caused by elevated ROS levels. Therefore, although no study to our best knowledge has shown a direct cause–effect relationship between Aβ-induced oxidative stress and alterations in the BBB in AD patients, it is likely that such changes are ROS-induced, eventually leading to BBB breakdown.

ROS have likewise been implicated in stroke. Stroke arises due to blockage of the cerebral blood vessels and is a major cause of death among the elderly [162]. Stroke can be generally classified as either ischemic (arising from occlusion of blood vessels) or haemorrhagic (arising from the bursting of blood vessels). A common secondary event in both types of stroke is brain oedema resulting from increased BBB permeability [143,163,164]. This elevated BBB permeability appears to stem from increased ROS levels. After all, it has been shown that superoxide production increases during subarachnoid haemorrhage [165]. Besides, levels of the antioxidants, Vitamin E and carotenoids were lower [166], while those of lipid oxidative damage products [167,168] were higher in ischemic stroke patients relative to controls, providing further evidence of oxidative stress in stroke.

It is plausible that increased ROS levels in stroke lead to the induction of MMP expression and activity. MMP-9 and MMP-2, in particular, were observed to be induced in ischemic rat brains [103]. As mentioned before, MMP activity is redox-regulated and has a major influence on BBB permeability. Besides, anti-MMP-9 neutralizing antibodies, like ROS scavengers, can decrease infarct size and reduce brain injury in animal models of stroke, implying a critical role for at least MMP-9 [169,170]. It is therefore likely that MMPs are key mediators of ROS-induced BBB breakdown in stroke.

Similar observations have been made in MS in which it has been proposed that ROS enhance the adhesion of monocytes to BMVECs, induce cytoskeletal rearrangement and alter TJ proteins, all of which lead to increased BBB permeability [144,171,172]. Severe oxidative damage to the major biomolecules, lipids, proteins and nucleotides in MS lesions has also been observed [173]. All these changes and damage are likely to both be a consequence of elevated ROS levels and to themselves contribute to BBB opening (see Figure 1). One group has also reported that Cu^{2+} chelation reduces MMP-9 activity by decreasing ROS production in MS lesions, thereby alleviating the clinical symptoms of experimental autoimmune encephalomyelitis (a mouse model of MS) [174]. Collectively, these studies suggest that ROS production is increased in MS, causing various changes such as MMP activation which eventually lead to BBB dysfunction.

Is antioxidant therapy effective in the treatment of neuropathologies?

Given the apparent involvement of ROS in neuropathology, a reasonable hypothesis would be that antioxidants, which reduce oxidative stress, can improve the prognosis of affected patients. However, the validity of this hypothesis is questionable. Taking stroke as an example, several trials have been conducted, both in humans and animals, in which various antioxidants were administered and outcome measures like mortality, infarct size and brain water were analysed. Studies in animal models have generally been encouraging. For instance, melatonin and edaravone, both free radical scavengers, reduced brain water content in rat models of subarachnoid haemorrhage [175,176]. Another study done in a rat model of ischemic stroke found that red wine polyphenolic compounds decreased brain infarct size [177]. All these studies appear to suggest that antioxidants improve prognosis in stroke. However, results from clinical trials have been less promising. For example, using disability and neurological deficits as outcome measures, NXY-059 (a potent free radical trapping agent) did not significantly alter prognosis in acute ischemic stroke [178]. Another antioxidant, tirilazad mesylate (a ROS scavenger and inhibitor of lipid peroxidation), actually increased the odds of being dead or disabled after an acute ischemic stroke, despite being an effective neuroprotectant in animal models [179]. Therefore, the effectiveness of antioxidant therapy in stroke cases remains uncertain.

Apart from stroke, the feasibility of antioxidant therapy has also been evaluated in animal models of TBI. The results appear promising with the Vitamin E analogue, MDL74, proving effective in attenuating TBI-induced cerebral oedema in rats with fluid percussion head injury [180]. A second study also found that treatment of TBI rats with propofol and erythropoietin (EPO), both known inhibitors of lipid peroxidation, reduced serum malondialdehyde levels, relative to untreated TBI rats [181]. In another study, rats on a diet supplemented with curcumin were found to suffer less TBI-associated cognitive impairment than rats on a regular diet [182]. Since curcumin is thought to be a better antioxidant-neuroprotectant than Vitamin E [183] and curcumin-supplemented rats suffered less oxidative damage than control rats [182], it appears that antioxidant therapy was effective as a prophylaxis in reducing TBI-induced neurological damage. However, as seen with the example of stroke, the ultimate test lies in clinical trials and the efficacy of antioxidant therapy in human TBI remains to be verified.

The inability to translate success in animal models to clinical cases could be due to several reasons, one of which is the ability to deliver the antioxidant to the site of injury within the brain and to do so at sufficiently high concentrations. One hypothesis put forth suggests that, in humans, damage to vessels supplying blood to the brain prevents delivery of the drug to the relevant tissues, thus leading to the failure of many clinical trials [184]. This could, for example, happen in ischemic stroke in which blood vessels are occluded. Failure to deliver high levels of

an antioxidant-drug to the brain is all the more critical as antioxidants evaluated clinically tend to be ROS scavengers and the high reactivity of ROS makes it necessary for the scavenger to be present at extremely high concentrations to effectively 'compete' with cellular molecules for reaction with ROS [185].

The time of administration of the antioxidant therapy following injury is also crucial to the success of a therapy. It has been suggested that the therapeutic window of antioxidants is narrow and that antioxidants may be effective only if administered within 1–2 h of injury [185]. Closer examination of the studies quoted above support this suggestion as studies in which benefits of antioxidant therapy were observed involved antioxidant administration within 2 h of injury [175–177]. In contrast, those studies which found no or even detrimental effects had longer time periods between injury and treatment of up to 24 h [178,179]. The narrow therapeutic window may be a reflection of the varying importance of ROS in different stages of injury, being particularly crucial only in the acute phase [184]. It may be that, beyond 2 h, events downstream of ROS have already set in and, thus, antioxidants can no longer influence outcome, resulting in the inefficacy of antioxidant therapy. The therapeutic window therefore is an extremely important factor in determining the effectiveness of a therapy [186] and the extended time periods inherent in the human trials may be a reason for the lack of effectiveness.

Besides, ROS can potentially be useful signalling molecules in injury repair and damage control. If antioxidant concentrations are too high, they may remove more ROS than is beneficial, thus impeding the initiation of repair processes, further exacerbating injury. For instance, although ROS-induced MMP-9 activity is associated with BBB breakdown, activated MMP-9 could possibly also function as a neuroprotectant by reducing the secretion of Aβ [187]. This could be important in limiting brain damage during AD in which Aβ plays a critical pathological role [188]. Therefore, excessive antioxidants may do more harm than good. To put it succinctly, antioxidants, as neuroprotectants, may simply have very narrow therapeutic ranges (in addition to short therapeutic windows) that vary between animals and humans and antioxidant concentrations evaluated in clinical trials may not fall within the clinical therapeutic range, resulting in failure of these trials.

It should also be noted that the perception of success in any study also depends on the outcome measures of that study. Endpoints measured in clinical trials such as disability and mortality [178,179] are usually influenced by multiple factors, not all of which may be significantly affected by the antioxidant therapy being evaluated. Therefore, even if the antioxidant improves one of the factors involved but leaves the others unchanged, outcome measures like mortality may remain severe and be without improvement, leading to a perception of failure when, in fact, the antioxidant was effective in ameliorating one aspect of the condition. Based on this, we suggest that combination therapy involving antioxidants and other neuroprotectants may lead to greater success in clinical trials than antioxidants used in isolation. Even focusing on antioxidants alone, it may be necessary to use multiple rather than single antioxidants for treatment. Taking vitamin E as an example, this antioxidant mainly protects against lipid oxidative damage. Singular use of vitamin E would therefore be largely ineffective against other forms of oxidative damage such as that to proteins and DNA which could have as important a pathological role as lipid peroxidation, if not more so. Therefore, because each antioxidant affords protection to a different sub-set of biomolecules, it may be necessary to administer combinations of antioxidants to provide all-round protection.

Even where antioxidant therapy has proven useful (e.g. in animal models where antioxidants improved stroke prognosis), it is unclear if the improvements observed were indeed due to an antioxidant effect. After all, antioxidants often possess non-antioxidant properties as well. For instance, melatonin influences apoptotic signalling and enhances the immune system, on top of being an antioxidant [189]. It could also modulate the cytoskeletal structure, for instance, by inducing the re-organization of actin filaments independent of an antioxidant effect [190]. Similarly, polyphenols, besides being antioxidants, are also capable of activating insulin signalling [191] and influencing transcription [192]. Furthermore, even in the studies where antioxidants proved to be effective therapy, reductions in markers of oxidative damage were not necessarily observed. For instance, while edaravone reduced oxidative DNA damage in the brain [176], melatonin did not reduce lipid peroxidation [175]. Since there is no clear consensus on the impact of antioxidant treatment on markers of oxidative damage, it is difficult to conclude definitively that any improvement observed must be due to an antioxidant effect.

Assuming an antioxidant mechanism to be necessary for clinical success, the usefulness of ROS scavengers may be limited given the stoichiometric nature of their ROS removal [184]. It may therefore be more prudent to develop approaches which overcome this limitation. For instance, Nrf-2 is a transcription factor which controls the expression of many antioxidant genes including catalase and SOD by binding to the antioxidant response element (ARE) upon dissociation from its inhibitor Keap1 [193]. Nrf-2 activation increases the resistance of neuronal cells and Nrf-2 knockout mice to oxidative stress-induced neurotoxicity [194]. The activation of

this transcription factor by sulphoraphane also reduces BBB leakiness due to brain injury [195], highlighting the potential of Nrf-2 as a therapeutic target [196]. Other possible strategies include the use of catalytic antioxidants [197] and metal chelators [198]. These approaches circumvent the limitations of conventional ROS scavengers in various ways. For instance, targeting Nrf-2 up-regulates endogenous antioxidant defences, generating enzymes like peroxidases that remove ROS enzymatically. Catalytic antioxidants function similarly, with each molecule of catalytic antioxidant removing multiple radicals, as opposed to traditional scavengers which remove ROS on a 1:1 ratio. Such strategies are therefore more efficient than the use of conventional ROS scavengers. Metal chelators, on the other hand, sequester redox-active metal ions like Cu^{2+} and Fe^{3+}, thereby preventing them from participating in reactions that generate ROS such as the Fenton reaction, thus eliminating ROS production by such means altogether.

Future directions

Although there is much evidence for the importance of ROS in neuropathology, the availability of effective antioxidant-neuroprotectants for human patients remains limited. As discussed above, there are several issues impeding the rapid development of an effective antioxidant therapy. For instance, the therapeutic window and efficacy range differs between drugs [186]. For a drug to be effective, it has to be administered within the right time frame at its therapeutic concentration and for a sufficient length of time from the point of injury. Therefore, any new candidate drug will have to be evaluated carefully to determine an appropriate therapeutic window and range.

In addition, where antioxidant therapy has proven effective, it is important to determine if the benefits were derived from an antioxidant effect or from other properties of the therapy. Knowledge of why a therapy is effective will aid in better drug design. For example, if the beneficial effects of a compound are due to its antioxidant properties, it may be economical to identify candidate drugs by screening compounds based on their antioxidant properties. Conversely, if the effective drugs improve prognosis independent of an antioxidant effect, such a strategy would be of no use.

The ability of candidate drugs to themselves cross the BBB is another important criterion in the search for effective neuroprotectants. The use of a drug for neuroprotection may be limited if it is unable to pass through the BBB [174], as that would preclude the drug from accessing the site of injury within the brain.

In summary, although there is little doubt of the involvement of ROS in neuropathology, it may be some time before an antioxidant therapy that is neuroprotective in humans can be found. Nonetheless, this is not to say that there are no candidate compounds currently available. For instance, *in vitro* and animal studies have been conducted with antioxidants like propofol [199], N-acetylcysteine and its derivative N-acetylcysteine amide [174,200] which have yielded promising results. Some of these are currently being evaluated in clinical trials, including EPO (for neurological outcome following TBI; trial number NCT00313716), propofol (also for head trauma; trial number NCT00336882) and edaravone (for stroke and head injury; ECCT-HIS) [201]. It is hopeful that as scientific knowledge of ROS and antioxidants in neurology improves, better and more effective neuroprotectants can be designed and applied to humans.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview structure, regulation and clinical implications. Neurobiol Dis 2004;16:1–13.
[2] Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, Tsuruo T, Sawada Y, Niwa M, Kataoka Y. Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-(production. Brain Res 2005;1038:208–215.
[3] Mooradian AD. Effect of aging on the blood-brain barrier. Neurobiol Aging 1988;9:31–39.
[4] Balda MS, Flores-Maldonado C, Cereijido M, Matter K. Multiple domains of occludin are involved in the regulation of paracellular permeability. J Cell Biochem 2000;78:85–96.
[5] Nitta T, Hata M, Gotch S, Seo Y, Sasaki H, Hashimoto N, Furuse H, Tsukita S. Size-selective loosening of the blood-brain barrier in claudin-5-deificent mice. J Cell Biol 2003; 161:653–660.
[6] Royo NC, Shimizu S, Schouten JW, Stover JF, McIntosh TK. Pharmacology of traumatic brain injury. Curr Opin Phamacol 2003;3:27–32.
[7] McQuaid S, Kirk J. The blood–brain barrier in multiple sclerosis. Int Congress Series 2005;1277:235–243.
[8] Heo JH, Han SW, Lee SK. Free radicals as triggers of brain edema formation after stroke. Free Radic Biol Med 2005; 39:51–70.
[9] Garcia Bueno B, Caso JR, Leza JC. Stress as a neuroinflammatory condition in brain: damaging and protective mechanisms. Neurosci Biobehav Rev 2008. doi: 10.1016/ j.neubiorev.2008.04.001.
[10] Olesen SP. Free oxygen radicals decrease electrical resistance of microvascular endothelium in brain. Acta Physiol Scand 1987;129:181–187.
[11] Agarwal R, Shukla GS. Potential role of cerebral glutathione in the maintenance of blood–brain barrier integrity in rat. Neurochem Res 1999;24:1507–1514.
[12] Kamiya T, Katayama Y, Kashiwagi F, Terashi A. The role of bradykinin in mediating ischemic oedema in rats. Stroke 1993;24:591–596.

[13] Relton J, Beckey V, Hanson W, Whalley E. CP-0597, a selective bradykinin B2 receptor antagonist, inhibits brain injury in a rat model of reversible middle cerebral artery occlusion. Stroke 1997;28:1430–1436.

[14] Prat A, Biernacki K, Pouly S, Nalbantoglu J, Couture R, Antel JP. Kinin B1 receptor expression and function on human brain endothelial cells. J Neuropathol Exp Neurol 2000;59:896–906.

[15] Bhattacharya SK, Rao PJ, Brumleve SJ, Paramar SS. Effects of intracerebroventricular administration of bradykinin on rat brain serotonin and prostaglandins. Res Commun Chem Path Pharmac 1986;54:355–366.

[16] Easton AS, Abbott NJ. Bradykinin increases permeability by calcium and 5-lipoxygenase in the ECV304/C6 cell culture model of the blood–brain barrier. Brain Res 2002;953: 157–169.

[17] Sarker MH, Fraser PA. Evidence that bradykinin increases permeability of single cerebral microvessels via free radicals. J Physiol 1994;479P:36P.

[18] Candelario-Jalil E, Taheri S, Yang Y, Sood R, Grossetete M, Estrada EY, Fiebich BL, Rosenberg GA. Cyclooxygenase inhibition limits blood–brain barrier disruption following intracerebral injection of tumor necrosis factor-α in the rat. J Pharmacol Exp Ther 2007;323:488–498.

[19] Moochhala SM, Lu J, Xing MC, Anuar F, Ng KC, Yang KL, Whiteman M, Atan S. Mercaptoethylguanidine inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expressions induced in rats after fluid-percussion brain injury. J Trauma 2005;59:450–457.

[20] Black KL, Hoff JT. Leukotrienes increase blood-brain barrier permeability in rats. Ann Neurol 1985;18:349–351.

[21] Minami T, Okazaki J, Kawabata A, Kawaki H, Okazaki Y, Tohno Y. Roles of nitric oxide and prostaglandins in the increased permeability of blood–brain barrier caused by lipopolysacharride. Envt Toxicol Pharmacol 1998;5:35–41.

[22] Rubinek T, Levy R. Arachidonic acid increases the activity of the assembled NADPH oxides in cytoplasmic membranes and endosomes. Biochim Biophys Acta Mol Cell Res 1993; 1176:51–58.

[23] Parpura V, Basarsky TA, Liu F, Jeftnija K, Jeftnija S, Haydon PG. Glutamate-mediated astrocytes-neuron signaling. Nature 1994;369:744–747.

[24] Schmidt H, Pollock JS, Nakane M, Forstermann U, Murad F. Ca2+/calmodulin-regulated nitric oxide synthases. Cell Calcium 1992;13:427–434.

[25] Nakano S, Matsukado K, Black KL. Increased brain tumour microvessel permeability after intracarotid bradykinin infusion is mediated by nitric oxide. Cancer Res 1996;56:4027–4031.

[26] Haorah J, Knipe B, Leibhart J, Ghorpade A, Persidsky Y. Alcohol-induced oxidative stress in brain endothelial cells causes blood–brain barrier dysfunction. J Leukoc Biol 2005; 78:1223–1232.

[27] Meyer DJ, Huxley VH. Capillary hydraulic conductivity is elevated by cGMP-dependent vasodilators. Circ Res 1992; 70:382–391.

[28] Yuan Y, Granger HJ, Zawieja DC, Chilian WM. Histamine increases venular permeability via a phospholipase C-NO synthase-guanylate cyclase cascade. Am J Physiol Heart Circ Physiol 1993;264:H1734–H1739.

[29] Myers DE, Larkins RG. Bradykinin-induced changes in phosphoinositides, inositol phosphate production and intracellular free calcium in cultured bovine aortic endothelial cells. Cell Signal 1989;1:335–343.

[30] Yuan SY. Protein kinase signaling in the modulation of microvascular permeability. Vasc Pharm 2002;39:213–223.

[31] Rathore R, Zheng Y, Niu C, Liu Q, Korde A, Ho Y, Wang Y. Hypoxia activates NADPH oxidase to increase $[ROS]_i$ and $[Ca^{2+}]_i$ through the mitochondrial ROS-PKC signaling axis in pulmonary artery smooth muscle cells. Free Radic Biol Med 2008. doi: 10.1016/j.freeradbiomed.2008.06.012.

[32] Xu H, Goettsch C, Xia N, Horke S, Morawietz H, Forstermann U, Li H. Differential roles of PKCα and PKCε in controlling the gene expression of Nox4 in human endothelial cells. Free Radic Biol Med 2008;44:1656–1667.

[33] Marshall LA, McCarte-Roshak A. Demonstration of similar calcium dependencies by mammalian high and low molecular mass phospholipase A2. Biochem Pharmacol 1992; 44:1849–1858.

[34] Kahles T, Luedike P, Endres M, Galla HJ, Steinmetz H, Busse R, Neumann-Haefelin T, Brandes RP. NADPH oxidase plays a central role in blood–brain barrier damage in experimental stroke. Stroke 2007;38:3000–3006.

[35] Deng X, Wang X, Andersson R. Influence of antiinflammatory and antioxidant agents on endothelial permeability alterations induced by bradykinin. J Invest Surg 1996; 9:337–349.

[36] Platt SR. The role of glutamate in central nervous system health and disease—a review. Vet J 2007;173:278–286.

[37] Sheldon AL, Robinson MB. The role of glutamate transporters in neurodegenerative diseases and the potential opportunities for intervention. Neurochem Int 2007;51: 333–355.

[38] Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of brain ischemic damage. Neuropharmacology 2008. doi: 10.1016/j.neuropharm.2008.01.005.

[39] Chen Z, Indyk JA, Bugge TH, Kombrinck KW, Degen JL, Strickland S. Neuronal death and blood–brain barrier breakdown after excitotoxic injury are independent processes. J Neurocsci 1999;19:9813–9820.

[40] Nitsch C, Hubauer H. Distant blood-brain barrier opening in subfields of the rat hippocampus after intrastriatal injections of kainic acid but not ibotenic acid. Neurosci Lett 1986;64:53–58.

[41] Emerich DF, Dean III RL, Bartus RT. The role of leukocytes following cerebral ischemia: pathogenic variable or bystander reaction to emerging infarct? Exp Neurol 2002;173:168–181.

[42] Hannah S, Mecklenburgh J, Rahman I, Bellingan GJ, Greening A, Haslett C, Chilvers ER. Hypoxia prolongs neutrophil survival *in vitro*. FEBS Lett 1995;372:233–237.

[43] Fabian RH, Kent TA. Superoxide anion production during reperfusion is reduced by an antineutrophil antibody after prolonged cerebral ischemia—effect of neutrophil depletion on extracellular ascorbyl radical formation. Free Radic Biol Med 1999;26:355–361.

[44] Ferrari CC, Depino AM, Prada F, Muraro N, Campbell S, Podhajcer O, Perry VH, Anthony DC, Pitossi FJ. Reversible demyelination, blood–brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain. Am J Pathol 2004;165:1827–1837.

[45] Bannister JV, Bellavite P, Davoli A, Thornalley PJ, Rossi F. The generation of hydroxyl radicals following superoxide production by neutrophil NADPH oxidase. FEBS Lett 1982;150:300–302.

[46] Bertram C, Misso NL, Fogel-Petrovic M, Figueroa C, Thompson PJ, Bhoola KD. Comparison of kinin B1 and B2 receptor expression in neutrophils of asthmatic and non-asthmatic subjects. Int Immunopharmacol 2007;7:1862–1868.

[47] Papini E, Grzeskowiak M, Bellavite P, Rossi F. Protein kinase C phosphorylates a component of NADPH oxidase of neutrophils. FEBS Lett 1985;190:204–208.

[48] Maridonneau-Parini I, Tauber AI. Activation of NADPH-oxidase by arachidonic acid involves phospholipase A2 in intact human neutrophils but not in the cell-free system. Biochem Biophys Res Comm 1986;138:1099–1105.

[49] Morgan L, Shah B, Rivers LE, Barden L, Groom AJ, Chung R, Higazi D, Desmond H, Smith T, Staddon JM. Inflammation and dephosphorylation of the tight junction protein occludin in an experimental model of multiple sclerosis. Neuroscience 2007;147:664–673.
[50] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335–344.
[51] Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR, Curi R. Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. J Physiol 2007;583:9–24.
[52] Swerdlow RH. Treating neurodegeneration by modifying mitochondria: potential solutions to a 'complex' problem. Antioxid Redox Signal 2007;9:1591–1603.
[53] Terzioglu M, Larsson NG. Mitochondrial dysfunction in mammalian ageing. Novartis Found Symp 2007;287:197–208.
[54] Murphy MP. How mitochondria produce reactive oxygen species. Biochem J 2009;417:1–13.
[55] Brouillet E, Jacquard C, Bizat N, Blum D. 3-nitropropinoic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. J Neurochem 2005;95:1521–1540.
[56] Coles CJ, Edmondson DE, Singer TP. Inactivation of succinate dehydrogenase by 3-nitropropionate. J Biol Chem 1979;254:5161–5167.
[57] Pandey M, Varghese M, Sindhu KM, Sreetama S, Navneet AK, Monhanakumar KP, Usha R. Mitochondrial NAD+-linked state 3 respiration and complex I activity are compromised in the cerebral cortex of 3-nitropropionic acid-induced rat model of Huntington's disease. J Neurochem 2008;104:420–434.
[58] Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. J Cell Sci 2007;120:4155–4166.
[59] Byun HO, Kim HY, Lim JJ, Seo YH, Yoon G. Mitochondrial dysfunction by complex II inhibition delays overall cell cycle progression via reactive oxygen species production. J Cell Biochem 2008;104:1747–1759.
[60] Kim GW, Gasche Y, Grzeschik S, Copin J, Maier CM, Chan PH. Neurodegeneration in striatum induced by the mitochondrial toxin 3-nitropropioninc acid: role of matrix metalloproteinase-9 in early blood–brain barrier disruption? J Neurosci 2003;23:8733–8742.
[61] Soares HD, Hicks RR, Smith D, McIntosh TK. Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. J Neurosci 1995;15:8223–8233.
[62] Bacher M, Weihe E, Dietzschold B, Meinhardt A, Vedder H, Gemsa D, Bette M. Borna disease virus-induced accumulation of macrophage migration inhibitory factor in rat brain astrocytes is associated with inhibition of macrophage infiltration. Glia 2002;37:291–306.
[63] Koshinaga M, Katayama Y, Fukushima M, Oshima H, Suma T, Takahata T. Rapid and widespread microglial activation induced by traumatic brain injury in rat brain slices. J Neurotrauma 2000;17:185–192.
[64] Bellander BM, Bendel O, Von Euler G, Ohlsson M, Svensson M. Activation of microglial cells and complement following traumatic injury in rat entorhinal-hippocampal slice cultures. J Neurotrauma 2004;21:605–615.
[65] Prat A, Biernacki K, Wosik K, Antel JP. Glial cell influence on the human blood–brain barrier. Glia 2001;36:145–155.
[66] Lynch NJ, Willis CL, Nolan CC, Roscher S, Fowler MJ, Weihe E, Ray DE, Schwaeble WJ. Microglial activation and increased synthesis of complement component C1q precedes blood-brain barrier dysfunction in rats. Mol Immunol 2004;40:709–716.
[67] Ryu JK, McLarnon JG. A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med 2008. doi: 10.111/j.1582-4934.2008.00434.x.
[68] Engel S, Schluesener H, Mittelbronn M, Seid K, Adjodah D, Wehner HD, Meyermann R. Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. Acta Neuropathol 2000;100:313–322.
[69] Dheen ST, Kaur C, Ling EA. Microglial activation and its implications in the brain diseases. Curr Med Chem 2007;14:1189–1197.
[70] Johnston RB Jr, Kitagawa S. Molecular basis for the enhanced respiratory burst of activated macrophages. Fed Proc 1985;44:2927–2932.
[71] Qian L, Gao X, Pei Z, Wu X, Block M, Wilson B, Hong JS, Flood PM. NADPH oxidase inhibitor DPI is neuroprotective at femtomolar concentrations through inhibition of microglia over-activation. Parkinsonism Relat Disord 2007;13(Suppl 3):S316–S320.
[72] Cheret C, Gervais A, Lilli A, Colin C, Amar L, Ravassard P, Mallet J, Cumano A, Krause KH, Mallat M. Neurotoxic activation of microglia is promoted by a nox1-dependent NADPH oxidase. J Neurosci 2008;28:12039–12051.
[73] Gehrmann J, Banati RB, Wiessner C, Hossmann KA, Kreutzberg GW. Reactive microglia in cerebral ischemia: an early mediator of tissue damage? Neuropathol Appl Neurobiol 1995;21:277–289.
[74] Kim HS, Cho IH, Kim JE, Shin YJ, Jeon JH, Kim Y, Yang YM, Lee KH, Lee JW, Lee WJ, Ye SK, Chung MH. Ethyl pyruvate has an anti-inflammatory effect by inhibiting ROS-dependent STAT signalling in activated microglia. Free Radic Biol Med 2008;45:950–963.
[75] Farber K, Cheung G, Mitchell D, Wallis R, Weihe E, Schwaeble W, Kettenmann H. C1q, the recognition subcomponent of the classical pathway of complement, drives microglial activation. J Neurosci Res 2008. doi: 10.1002/jnr.21875.
[76] Jorens PG, Matthys KE, Bult H. Modulation of nitric oxide synthase activity in macrophages. Mediators Inflamm 1995;4:75–89.
[77] Marques CP, Cheeran MC, Palmquist JM, Hu S, Lokensgard JR. Microglia are the major cellular source of inducible nitric oxide synthase during experimental herpes encephalitis. J Neurovirol 2008;14:229–238.
[78] Stelzner TJ, Weil JV, O'Brien RF. Role of cyclic adenosine monophosphate in the induction of endothelial barrier properties. J Cell Physiol 1989;139:157–166.
[79] Hall NC, Carrey JM, Plante OJ, Chang M, Butterfield DA. Effect of 2-cyclohexane-1-one induced glutathione diminution on ischemia/reperfusion-induced alterations in the physical state of brain synaptosomal membrane protein and lipids. Neurosicence 1997;77:283–290.
[80] Huang CF, Hsu CJ, Liu SH, Lin-Shiau SY. Neurotoxicological mechanism of methylmercury induced by low-dose and long-term exposure in mice: oxidative stress and down-regulated Na+/K+-ATPase involved. Toxicol Lett 2008;176:188–197.
[81] Mertsch K, Blasig I, Grune T. 4-hydroxynonenal impairs the permeability of an *in vitro* rat blood–brain barrier. Neurosci Lett 2001;314:135–138.
[82] Shi F, Cavitt J, Audus KL. 21-aminosteroid and 2-aminomethyl chromans inhibition of arachnoid acid-induced lipid peroxidation and permeability enhancement in bovine brain microvessel endothelial cell monolayers. Free Radic Biol Med 1995;19:349–357.

[83] Smith SL, Scherzh HM, Hall ED. Protective effects of tirilazad mesylate and metabolite -89678 against blood-brain barrier damage after subarachnoid hemorrhage and lipid peroxidative neuronal injury. Neurosurgery 1996;84: 229–233.

[84] Lim SO, Gu JM, Kim MS, Kim HS, Park YN, Park CK, Cho JW, Park YM, Jung G. Epigenetic changes induced by reactive oxygen species in hepatocellular carcinoma: methylation of the E-cadherin promoter. Gastroenterology 2008; 135:2128–2140.

[85] Abbruscato TJ, Davis TP. Protein expression of brain endothelial cell E-cadherin after hypoxia/aglycemia: influence of astrocyte contact. Brain Res 1999;842:277–286.

[86] Pal D, Audus KL, Siahaan TJ. Modulation of cellular adhesion in bovine brain microvessel endothelial cells by a decapeptide. Brain Res 1997;747:103–113.

[87] Reese TS, Karnovsky MJ. Fine structural localization of a blood–brain barrier to exogenous peroxidase. J Cell Biol 1967;34:207–217.

[88] Krizbai IA, Bauer H, Bresgen N, Eckl PM, Farkas A, Szatmari E, Traweger A, Wejksza K, Bauer HC. Effect of oxidative stress on the junctional proteins of cultured cerebral endothelial cells. Cell Mol Neurobiol 2005; 25:129–139.

[89] Schreibelt G, Kooij G, Reijerkerk A, van Dooren R, Gringhuis SI, van der Pol S, Weksler BB, Romero IA, Couraud PO, Piontek J, Blasig UE, Dijkstra CD, Ronken E, de Vries HE. Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3-kinase and PKB signaling. FASEB J 2007;21:3666–3676.

[90] Lee H, Namkoong K, Kim D, Kim K, Cheong Y, Kim S, Lee W, Kim K. Hydrogen peroxide-induced alterations of tight junction proteins in bovine brain microvascular endothelial cells. Microvasc Res 2004;68:231–238.

[91] Ishizaki T, Chiba H, Kojima T, Fujibe M, Soma T, Miyajima H, Nagasawa K, Wada I, Sawada N. Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood–brain barrier endothelial cells via protein kinase A-dependent and -independent pathways. Exp Cell Res 2003;290:275–288.

[92] Wong V. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. Am J Physiol 1997;273:C1859–C1867.

[93] Schreibelt G, Musters RJP, Reijerkerk A, de Groot LR, van der Pol SMA, Hendrikx EML, Dopp ED, Dijkstra CD, Drukarch B, de Vries HE. Lipoic acid affects cellular migration into the central nervous system and stabilizes blood–brain barrier integrity. J Immunol 2006;177:2630–2637.

[94] Ridley AJ, Hall A. Signal transduction pathways regulating Rho-mediated stress fibre formation: requirement for a tyrosine kinase. EMBO J 1994;13:2600–2610.

[95] Pellegrin S, Mellor H. Actin stress fibres. J Cell Sci 2007; 120:3491–3499.

[96] Shiu C, Barbier E, Di Cello F, Choi HJ, Stins M. HIV-1 gp120 as well as alcohol affect blood–brain barrier permeability and stress fiber formation: involvement of reactive oxygen species. Alcohol Clin Exp Res 2007;31:130–137.

[97] Hirase T, Kawashima S, Wong E, Uemada T, Rikitake Y, Tsukita S, Yokoyama M, Staddon J. Regulation of tight junction permeability and occludin phosphorylation by RhoAp160ROCK-dependent and independent mechanism. J Biol Chem 2001;276:10423–10431.

[98] Reijerkerk A, Kooij G, van der Pol SMA, Khazen S, Dijkstra CD, de Vries HE. Diapedesis of monocytes is associated with MMP-mediated occludin disappearance in brain endothelial cells. FASEB J 2006;20:E1901–E1909.

[99] Haorah J, Ramirez SH, Schall K, Smith D, Pandya R, Persidsky Y. Oxidative stress activates protein tyrosine kinase and matrix metalloproteinases leading to blood–brain barrier dysfunction. J Neurochem 2007;101:566–576.

[100] Maier CM, Hsieh L, Crandall T, Narasimhan P, Chan PH. A new approach for the investigation of reperfusion-related brain injury. Biochem Soc Trans 2006;34:1366–1369.

[101] Morita-Fujimura Y, Fujimura M, Gasche Y, Copin JC, Chan PH. Overexpression of copper and zinc superoxide dismutase in transgenic mice prevents the induction and activation of matrix metalloproteinases after cold injury-induced brain trauma. J Cerebral Blood Flow Metab 2000; 20:130–138.

[102] Nakashima J, Jato M, Akhand A, Suzuki H, Takeda K, Hossain K, Kawamoto Y. Redox-linked signal transduction pathways for protein tyrosine kinase activation. Antioxid Redox Signal 2002;4:517–531.

[103] Rosenberg GA, Estrada E, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke 1998;29:2189–2195.

[104] Kim S, Bae Y, Bae S, Choi K, Yoon K, Koo T, Jang H, Yun I, Kim K, Kwon Y, Yoo M, Bae M. Visfatin enhances ICAM-1 and VCAM-1 expression through ROS-dependent NF-κB activation in endothelial cells. Biochim Biophys Acta Mol Cell Res 2008;1783:886–895.

[105] Etienne-Manneville S, Manneville JB, Adamson P, Wilbourn B, Greenwood J, Couraud PP. ICAM-1-coupled cytoskeleton rearrangements and transendothelial lymphocyte migration involves intracellular calcium signaling in brain endothelial cell lines. J Immunol 2000;165:3375–3383.

[106] Fabene PF, Mora GN, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constatin G. role for leukocyte-endothelial adhesion mechanisms in epilepsy. Nat Med 2008;14:1377–1383.

[107] Adamson P, Etienne S, Courand PO, Calder V, Greenwood J. Lymphocyte migration through brain endothelial cell monolayers involves signaling through endothelial ICAM-1 via a Rho-dependent pathway. J Immunol 1999;162:2964–2973.

[108] Pinsky DJ, Yan S, Lawson C, Naka Y, Chen J, Connolly Jr ES, Stern DM. Hypoxia and modification of the endothelium: implications for regulation of vascular homeostatic properties. Sem Cell Biol 1995;6:283–294.

[109] Eugenin EA, Berman JW. Chemokine-dependent mechanisms of leukocyte trafficking across a model of the blood–brain barrier. Methods 2003;29:351–361.

[110] Doring A, Wild M, Vestwebeer D, Deutsch U, Engelhardt B. E- and P-selectin are not required for the development of experimental autoimmune encephalomyelitis in C57BL/6 and SJL mice. J Immunol 2007;179:8470–8479.

[111] Roy A, Jana A, Yatish K, Freidt MB, Fung YK, Martinson JA, Pahan K. Reactive oxygen species up-regulate CD11b in microglia via nitric oxide: implications for neurodegenerative disease. Free Radic Biol Med 2008;45:686–699.

[112] Tsukimori K, Tsushima A, Fukushima K, Nakano H, Wake N. Neutrophil-derived reactive oxygen species can modulate neutrophil adhesion to endothelial cells in preeclampsia. Am J Hypertens 2008;21:587–591.

[113] Rattan V, Sultana C, Shen Y, Kalra VK. Oxidant stress-induced transendothelial migration of monocytes is linked to phosphorylation of PECAM-1. Am J Physiol 1997;273: E453–E461.

[114] Shen JS, Meng XL, Moore DF, Quirk JM, Shayman JA, Schiffmann R, Kaneski CR. Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule

expression in Fabry disease endothelial cells. Mol Genet Metab 2008;95:163–168.

[115] Takano M, Meneshian A, Sheikh E, Yamakawa Y, Wilkins KB, Hopkins EA, Bulkley GB. Rapid upregulation of endothelial P-selectin expression via reactive oxygen species generation. Am J Physiol Heart Circ Physiol 2002;283: H2054–H2061.

[116] Eugenin EA, Gamss R, Buckner C, Buono D, Klein RS, Schoenbaum EE, Calderon TM, Berman JW. Shedding of PECAM-1 during HIV infection: a potential role for soluble PECAM-1 in the pathogenesis of NeuroAIDS. J Leukoc Biol 2006;79:444–452.

[117] Coisne C, Faveeuw C, Delplace Y, Dehouck L, Miller F, Cecchelli R, Dehouck B. Differential expression of selectins by mouse brain capillary endothelial cells *in vitro* in response to distinct inflammatory stimuli. Neurosci Lett 2006; 392:216–220.

[118] Cayrol R, Wosik K, Berard JL, Deodelet-Devillers A, Ifergan I, Kebir H, Haggani AS, Kreymborg K, Krug S, Moumdjian R, Bouthillier A, Becher B, Arbour N, David S, Stanimirovic D, Prat A. Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. Nat Immunol 2007;9:137–145.

[119] Brandes RP, Miller FJ, Beer S, Haendeler J, Hoffmann J, Ha T, Holland SM, Gorlach A, Busse R. The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression. Free Radic Biol Med 2002;32:1116–1122.

[120] Wang FS, Wang CJ, Chen YJ, Chang PR, Huang YT, Sun YC, Huang HC, Yang YJ, Yang KD. Ras induction of superoxide activates ERK-dependent angiogenic transcription factor HIF-1 and VEGF-A expression in shock wave-stimulated osteoblasts. J Biol Chem 2004;279:10331–10337.

[121] Wang W, Dentler WL, Borchardt RT. VEGF increases BMEC monolayer permeability by affecting occluding expression and tight junction assembly. Am J Physiol Heart Circ Physiol 2001;280:H434–H440.

[122] DeMaio L, Rouhanizadeh M, Reddy S, Sevanian A, Hwang J, Hsiai TK. Oxidized phospholipids mediate occluding expression and phosphorylation in vascular endothelial cells. Am J Physiol Heart Circ Physiol 2006;290:H674–H683.

[123] Wang W, Merrill MJ, Borchardt RT. Vascular endothelial growth factor affects permeability of brain microvessel endothelial cells *in vitro*. Am J Physiol Cell Physiol 1996; 271:1973–1980.

[124] Lagrange P, Romero IA, Minn A, Revest PA. Transendothelial permeability changes induced by free radicals in an *in vitro* model of the blood–brain barrier. Free Radic Biol Med 1999;27:667–672.

[125] Noble LJ, Maida N, Igarashi T. The blood-brain barrier after traumatic injury: vascular and parenchymal interactions. In: Sharma H. Blood-Spinal Cord and Brain Barriers in Health and Disease. 1st ed. Academic Press, Incorporated; 2004. pp. 419–435.

[126] Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell 2002;9:387–399.

[127] Valen G, Sonden A, Vaage J, Malm E, Kjellstrom BT. Hydrogen peroxide induces endothelial cell atypia and cytoskeleton depolymerization. Free Radic Biol Med 1999; 26:1480–1488.

[128] Kontos CD, Wei EP, Williams JI, Kontos HA, Povlishock JT. Cytochemical detection of superoxide in cerebral inflammation and ischemia *in vivo*. Am J Physiol 1992;263(Heart Circ Physiol 32):H1234–H1242.

[129] Nelson CW, Wei EP, Povlishock JT, Kontos HA, Moskowitz MA. Oxygen radicals in cerebral ischemia. Am J Physiol 1992;253(Heart Circ Physiol32):H1356–H1362.

[130] Mayhan WG. Nitric oxide donor-induced increase in permeability of the blood–brain barrier. Brain Res 2000; 866:101–108.

[131] Hurst RD, Clark JB. Nitric oxide-induced blood-brain barrier dysfunction is not mediated by inhibition of mitochondrial respiratory chain activity and/or energy depletion. Nitric Oxide Biol Chem 1997;1:121–129.

[132] Boje KMK, Lakhman SS. Nitric oxide redox species exert differential permeability effects on the blood–brain barrier. J Pharmacol Exp Ther 2000;293:545–550.

[133] Utepbergenov DI, Mertsch K, Sporbert A, Tenz K, Paul M, Haseloff RF, Blasig IE. Nitric oxide protects blood–brain barrier in vitro from hypoxia/re-oxygenation-mediated injury. FEBS Lett 1998;424:197–201.

[134] Nathan C, Xie Q. Regulation of biosynthesis of nitric oxide. J Biol Chem 1994;269:13725–13728.

[135] Scott GS, Bowman SR, Smith T, Flower RJ, Bolton C. Glutamate-stimulated peroxynitrite production in a brain-derived endothelial cell line is dependent on N-methyl-D-aspartate (NMDA) receptor activation. Biochem Pharmacol 2007;73:228–236.

[136] Phares TW, Fabis MJ, Brimer CM, Kean RB, Hooper DC. A peroxynitrite-dependent pathway is responsible for blood–brain barrier permeability changes during a central nervous system inflammatory response: TNF-α is neither necessary nor sufficient. J Immunol 2007;178:7334–7343.

[137] Kuhlmann CRW, Tamaki R, Gamerdinger M, Lessmann V, Behl C, Kempski O, Luhmann HJ. Inhibition of the myosin light chain kinase prevents hypoxia-induced blood–brain barrier disruption. J Neurochem 2007;102:501–507.

[138] Guix FX, Uribesalgo I, Coma M, Munoz FJ. The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 2005;76:126–152.

[139] Winkler F, Koedel U, Kastenbauer S, Pfister HW. Differential expression of nitric oxide synthases in bacterial meningitis: role of the inducible isoform for blood–brain barrier breakdown. J Infect Dis 2001;183:1749–1759.

[140] Lu J, Moochhala S, Kaur C, Ling EA. Cellular inflammatory response associated with breakdown of the blood–brain barrier after closed head injury in rats. J Neurotrauma 2001; 18:399–408.

[141] Boje KMK. Inhibition of nitric oxide synthase attenuates blood–brain barrier disruption during experimental meningitis. Brain Res 1996;720:75–83.

[142] Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 1997;23:134–147.

[143] Menzies SA, Betz AL, Hof JT. Contribution of ions and albumin to the formation and resolution of ischemic brain edema. J Neurosurg 1993;78:257–266.

[144] Kirk J, Plumb J, Mirakhur M, McQuaids S. Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood–brain barrier leakage and active demyelination. J Pathol 2003;201:319–327.

[145] Algotsson A, Winblad B. The integrity of the blood–brain barrier in Alzheimer's disease. Acta Neurol Scand 2007; 115:403–408.

[146] Farrall AJ, Wardlaw JM. Blood–brain barrier: ageing and microvascular disease—systematic review and meta-analysis. Neurobiol Aging 2007. doi: 10.1016/j.neurobiolaging. 2007.07.015.

[147] Ujiie M, Dickstein DL, Carlow DA, Jefferies WA. Blood–brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. Microcirculation 2003; 10:463–470.

[148] Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. Blood–brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology 2007;68:1809–1814.

[149] Gonzalez-Velasquez FJ, Kotarek JA, Moss MA. Soluble aggregates of the amyloid-beta protein selectively stimulate permeability in human brain microvascular endothelial monolayers. J Neurochem 2008;107:466–477.

[150] Li G, Ma R, Huang C, Tang Q, Fu Q, Liu H, Hu B, Xiang J. Protective effect of erythropoietin on beta-amyloid-induced PC12 cell death through antioxidant mechanisms. Neurosci Lett 2008;442:143–147.

[151] Zhou J, Zhang S, Zhao X, Wei T. Melatonin impairs NADPH oxidase assembly and decreases superoxide anion production in microglia exposed to amyloid-beta1-42. J Pineal Res 2008;45:157–165.

[152] Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. J Neurochem 1997;68:2061–2069.

[153] Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J Neurochem 2005;93: 953–962.

[154] Smith DG, Cappai R, Barnham KJ. The redox chemistry of the Alzheimer's disease amyloid beta peptide. Biochim Biophys Acta 2007;1768:1976–1990.

[155] Chen JX, Yan SD. Amyloid-beta-induced mitochondrial dysfunction. J Alzheimers Dis 2007;12:177–184.

[156] Sipos I, Tretter L, Adam-Vizi V. Quantitative relationship between inhibition of respiratory complexes and formation of reactive oxygen species in isolated nerve terminals. J Neurochem 2005;84:112–118.

[157] De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 2007;282:11590–11601.

[158] Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY. Amyloid beta peptide and NDMA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. J Neurochem 2008;106:45–55.

[159] Marco S, Skaper SD. Amyloid beta-peptide1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells. Neurosci Lett 2006;401:219–224.

[160] Leake C, Morris CM, Whateley J. Brain matrix metalloproteinase 1 levels are elevated in Alzheimer's disease. Neurosci Lett 2000;291:201–203.

[161] Lorenzl S, Albers DS, Relkin N, Ngyuen T, Hilgenberg SL, Chirichigno J, Cudkowicz ME, Beal MF. Increased plasma levels of matrix metalloproteinase-9 in patients with Alzheimer's disease. Neurochem Int 2003;43:191–196.

[162] Bronner LL, Kunter DS, Mansom JE. Primary prevention of stroke. N Eng J Med 1995;333:1392–1400.

[163] Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. Stroke 1990;21:801–807.

[164] Rosenberg GA, Estrada E, Kelley RD, Kornfeld M. Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rats. Neurosci Lett 1993;160: 117–119.

[165] Mori T, Nagata K, Town T, Tan J, Mathi T, Asano T. Intracisternal increase of superoxide anion production in a canine subarachnoid haemorrhage model. Stroke 2001;32: 636–642.

[166] Chang CY, Lai YC, Cheng TJ, Lau MT, Hu ML. Plasma levels of antioxidant vitamins, selenium, total sulfhydryl groups and oxidative products in ischemic-stroke patients as compared to matched controls in Taiwan. Free Radic Res 1998;28:15–24.

[167] Ferretti G, Bacchetti T, Masciangelo S, Nanetti L, Mazzanti L, Silvestrini M, Bartolini M, Provinciali L. Lipid peroxidation in stroke patients. Clin Chem Lab Med 2008;46: 113–117.

[168] Kelly PJ, Marrow JD, Nig M, Kovoshetz W, Lo EH, Terry E, Milne GI, Hubbard J, Lee H, Stevenson E, Lederer M, Furie KL. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the Biomarker Evaluation for Antioxidant Therapies in Stroke (BEAT-Stroke) study. Stroke 2008;39:100–104.

[169] Rak R, Chao DL, Pluta RM, Mitchell J, Olfield EH, Watson JC. Neuroprotection by the stable nitroxide Tempol during reperfusion in a rat model of transient focal ischemia. J Neurosurg 2000;92:646–657.

[170] Rak R, Chao DL, Pluta RM, Mitchell J, Olfield EH, Watson JC. Neuroprotection by the stable nitroxide Tempol during reperfusion in a rat model of transient focal ischemia. J Neurosurg 2000;92:646–657.

[171] van der Goes A, Wouters D, van der Pol S, Huizinga R, Ronken E, Adamson P, Greenwood J, Dijksta CD, de Vries HE. Reactive oxygen species enhance the migration of monocytes across the blood–brain barrier *in vitro.* FASEB J 2001;15:1852–1854.

[172] Mark KS, Davis TP. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. Am J Physiol Heart Care Physiol 2002;282: H1485–H1494.

[173] van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra CD, van der Valk P, de Vries HE. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radic Biol Med 2008;45: 1729–1737.

[174] Offen D, Gilgun-Sherki Y, Barhum Y, Benhar M, Grinberg L, Reich R, Melamed E, Atlas D. A low molecular weight copper chelator crosses the blood–brain barrier and attenuates experimental autoimmune encephalomyelitis. J Neurochem 2004;89:1241–1251.

[175] Ayer RE, Sugawara T, Chen W, Tong W, Zhang JH. Melatonin decreases mortality following severe subarachnoid hemorrhage. J Pineal Res 2008;44:197–204.

[176] Nakamura T, Kuroda Y, Yamashita S, Zhang X, Miyamoto O, Tamiya T, Nagao S, Xi G, Keep RF, Itano T. Edaravone attenuates brain edema and neurologic deficits in a rat model of acute intracerebral hemorrhage. Stroke 2008;39: 463–469.

[177] Ritz M, Ratajczak P, Curin Y, Cam E, Mendelowitsch A, Pinet F, Andriantsitohaina R. Chronic treatment with red wine polyphenol compounds mediates neuroprotection in a rat model of ischemic cerebral stroke. J Nutr 2008;138: 519–525.

[178] Diener HC, Less KR, Lyden P, Grotta J, Davalos A, Davis SM, Shuaib A, Ashwood T, Wasiewski W, Alderfer V, Hardemark H, Rodichok L. NXY-059 for the treatment of acute stroke—pooled analysis of the SAINT I and II trials. Stroke 2008;39:1751–1758.

[179] The Tirilazad International Steering Committee, Tirilazad for acute ischemic stroke. Cochrane Database of Systematic Reviews 2001;4. Art No: CD002087. doi: 10.1002/ 14651858.CD002087.

[180] Petty MA, Poulet P, Haas A, Namer IJ, Wagner J. Reduction of traumatic brain injury-induced cerebral oedema by a free radical scavenger. Eur J Pharmacol 1996;307:149–155.

[181] Ozturk E, Demirbilek S, Kadir But A, Saricicek V, Gulec M, Akyol O, Ozcan Ersoy M. Antioxidant properties of propofol and erythropoietin after closed head injury in rats. Prog Neuro Psychopharmacol Biol Psych 2005;29:922–927.

[182] Wu A, Ying Z, Gomez-Pinilla F. Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative

stress, synaptic plasticity and cognition. Exp Neurol 2006; 197:309–317.
[183] Martin-Aragon S, Benedi JM, Villar AM. Modifications on antioxidant capacity and lipid peroxidation in mice under fraxetin treatment. J Pharm Pharmacol 1997;49:49–52.
[184] Fagan SC, Hess DC, Hohnadel EJ, Pollock DM, Ergul A. Targets for vascular protection after acute ischemic stroke. Stroke 2004;35:2220–2225.
[185] Kontos HA. Oxygen radicals in cerebral ischemia: the 2001 Willis lecture. Stroke 2001;32:2712–2716.
[186] Hsu CY, Ahmed SH, Less KR. The therapeutic time window—theoretical and practical considerations. J Stroke Cerbrovasc Dis 2000;9:24–31.
[187] Talamagas AA, Efthimiopoulos S, Tsilibary EC, Figueiredo-Pereira ME, Tzinia AK. Abeta(1-40)-induced secretion of matrix metalloproteinase-9 results in sAPPalpha release by association with cell surface APP. Neurobiol Dis 2007; 28:304–315.
[188] Walsh DM, Selkoe DJ. A beta oligomers: a decade of discovery. J Neurochem 2007;101:1172–1184.
[189] Pandi-Perumal SR, Srinivasan V, Maaestroni GJM, Cardinali DP, Poeggeler B, Hardeland R. Melatonin: nature's most versatile biological signal? FEBS J 2006;273:2813–2838.
[190] Benitez-King G. Melatonin as a cytoskeletal modulator: implications for cell physiology and disease. J Pineal Res 2006;40:1–9.
[191] Cameron AR, Anton S, Melville L, Houston NP, Dayal S, McDougall GJ, Stewart D, Rena G. Black tea polyphenols mimic insulin-insulin-like growth factor-1 signaling to the longevity factor FOXO1a. Aging Cell 2008;7:69–77.
[192] Fresco P, Borges F, Diniz C, Marques MPM. New insights on the anticancer properties of dietary polyphenols. Med Res Rev 2006;26:747–766.
[193] Kobayahi M, Yamamoto M. Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul 2006;46:113–140.
[194] Johnson JA, Johnson DA, Kraft AD, Calkins MJ, Jakel RJ, Vargas MR. The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegenration. Ann NY Acad Sci 2008;1147:61–69.
[195] Zhao J, Moore AN, Redell JB, Dash PK. Enhancing expression of Nrf2-driven genes protects the blood-brain barrier after brain injury. J Neurosci 2007;27:10240–10248.
[196] de Vries HE, Witte M, Hondius D, Rozemuller AJM, Drukarch B, Hoozermans J, van Horssen J. Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenrative disease? Free Radic Biol Med 2008;45:1375–1383.
[197] Golden TR, Patel M. Catalytic antioxidants and neurodegeneration. Antioxid Redox Signal 2008. doi: 10.1089/ars.2008.2256.
[198] Hague T, Andrews PL, Barker J, Naughton DP. Dietary chelators as antioxidant enzyme mimetics: implications for dietary intervention in neurodegenerative diseases. Behav Pharmacol 2006;17:425–430.
[199] Sagara Y, Hendler S, Khoh-Reiter S, Gillenwater G, Carlo D, Schubert D, Chang J. Propofol hemisuccinate protects neuronal cells from oxidative injury. J Neurochem 1999;73: 2524–2530.
[200] Price TO, Uras F, Banks WA, Ercal N. A novel antioxidant N-acetylcysteine-amide prevents gp120- and Tat- induced oxidative stress in brain endothelial cells. Exp Neurol 2006; 201:193–202.
[201] ClinicalTrials.gov Database [Internet]. National Institutes of Health, Department of Health and Human Services (US). Available online at: http://clinicaltrials.gov/ct2/home, accessed 20 June 2008.

This paper was first published online on iFirst on 24 February 2009.