Molecular biological considerations in cerebral vasospasm following aneurysmal subarachnoid hemorrhage

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Chronic delayed cerebral vasospasm (CDCV) remains a serious and often fatal complication of aneurysmal subarachnoid hemorrhage (SAH). The current understanding of its fundamental mechanisms and molecular biological characterization is rudimentary. Two important vasoactive substances have been implicated in CDCV: endothelin-1 (ET-1) and nitric oxide (NO). A 21-amino acid vasoconstrictor peptide, ET-1 has generated interest as a possible important contributor to cerebral vasospasm on the basis of both clinical and experimental evidence suggesting abnormally enhanced production. Nitric oxide is a cell membrane-permeable free radical gas that accounts for the vasodilatory effect of endothelium-derived relaxation factor and is a physiological antagonist of ET-1. As with ET-1, abnormalities of NO production have been implicated in several pathological conditions including cerebral vasospasm. This brief report reviews some of the physiological and regulatory features of these two molecules and explores the possibility of their relationship to cerebral vasospasm.

Key Words * nitric oxide * endothelin-1 * vasospasm * subarachnoid hemorrhage

The chronic delayed type of cerebral vasoconstriction that characterizes vasospasm associated with aneurysmal subarachnoid hemorrhage (SAH) remains the most important cause of mortality and morbidity in those patients surviving an initial SAH.[2,24,30] Many possible causes for the condition have been considered over the past 30 years. Particular interest in erythrocytes (red blood cells [RBCs]), was generated by studies that distinguished acute from chronic delayed cerebral vasospasm (CDCV).[11,12,33,55,56,65,76]

Several facts related to RBCs are noteworthy with regard to CDCV. In studies RBCs were observed to lyse after a period of hours to days, and oxyhemoglobin released during these events was implicated as a cause.[8,42,69] The time course of RBC lysis was consistent with the clinical delay that characterizes CDCV,[74] and the release of oxyhemoglobin from the RBCs during lysis was also consistent with the apparent relationship of CDCV to the volume of SAH.[15] Furthermore, some evidence was offered for the effectiveness of removing blood from the subarachnoid space in warding off CDCV.[21,53,61] The molecular link between oxyhemoglobin and vasospasm, however, remains unknown.

ENDOTHELIN-1
The discovery of endothelin-1 (ET-1) in 1988 provided one of the first important opportunities to explore the pathophysiological mechanisms of CDCV in molecular biological terms.[78] The endothelins were subsequently found to be a family of genes specifying short peptides with diverse functions. Genes for three subtypes of endothelin were mapped and characterized.[79] Endothelin-1 was determined to have very strong vasomotor activity. The ET-1 gene specifies a 21-amino acid peptide product with several disulfide linkages and potent vasoconstrictor activity restricted to its end-terminal hexapeptide.[78] Its structure very closely resembles that of the snake venom toxin of Atractaspis engaddensis, a Middle Eastern asp whose bite rapidly kills rodents by inducing severe coronary vasoconstriction and is lethal in minute amounts.[36,50,68] Endothelin-1 is the most potent vasoconstrictor known and is approximately 10 times as potent as angiotensin II.[78] It is present in many diverse species and has been highly conserved in evolution (porcine ET-1 is identical to human, for example), which implies its biological importance.

Endothelin-1 effects vasoconstriction by a receptor-mediated mechanism that releases Ca++ from intracellular stores and also activates Ca++ entry via dihydropyridine-sensitive voltage-gated Ca++ channels, although the latter (which is the target of nimodipine) does not appear to be a direct effect.[75] The specific binding pattern of ET is not susceptible to Ca++ channel blockade.[37] Two specific types of G-protein-coupled receptor have been identified, characterized, and cloned.[62,63] The ET-A receptor is specifically activated by ET-1 and is present on vascular smooth-muscle cells as well as endothelial cells.[40,59] Of importance, and perhaps particularly relevant to CDCV, ET-1 has been well demonstrated to act from the adventitial side.[45,77] Several signal transduction mechanisms linked to receptor activation have been identified.[62,63] Endothelin-1 messenger RNA is extremely unstable and its half-life is short; ET-1 is rapidly eliminated from the bloodstream.[79] The biosynthesis of ET-1 is regulated at the level of transcription.[78] These and other findings indicate that its action is predominantly local.[41] Once bound, however, its dissociation from the receptor is extremely slow, and the vasoconstriction that it causes is characteristically resistant to washout.[79]

**NITRIC OXIDE**

Nitric oxide (NO) began to generate substantial scientific interest among biochemists and vascular physiologists during the late 1970s, when it was proposed as the common intermediate to a class of drugs that caused smooth-muscle relaxation in association with increasing cyclic guanosine monophosphate (cGMP) levels.[3,49] The term "nitrovasodilators" was coined by Murad[49] in this context. Furchgott and Zawadzki, on the basis of earlier work,[18] proposed that the substance derived from endothelium, known as endothelium-derived relaxation factor (EDRF), that was responsible for the phenomenon of vasodilation by agents like acetylcholine, histamine, and bradykinin (agents causing vasoconstriction when the endothelium was removed), was in fact NO. Research interest in this molecule grew exponentially thereafter. More than 4000 publications on NO appeared in 1996,[49] and there is no sign of waning of interest. The more that is discovered about this molecule, the more fascinating it becomes. The smallest known biologically active molecule, NO is a cell membrane-permeable free radical gas that fully accounts for the vasodilator activity of EDRF and is a physiological antagonist of ET-1.[57] Nitric oxide interacts with several intracellular targets including soluble guanylate cyclase, stimulating the conversion of guanosine triphosphate to cGMP. Cyclic guanosine monophosphate activates cGMP-dependent protein kinases leading to vascular smooth-muscle relaxation and inhibition of platelet adhesion and aggregation.[23,64] Thus NO is a powerful vasodilator. Its known functions are extremely diverse, however, and include maintenance of basal vasomotor tone, platelet inhibition,
macrophage-mediated cytotoxicity, neurotransmission, synaptogenesis, intercellular signaling, N-methyl-D-aspartate-mediated neuronal excitotoxicity, selective neuroprotection, penile erection, apoptosis, nonadrenergic noncholinergic intestinal relaxation, and free radical generation.[51,64] The pathological roles are as well-represented as the physiological ones, earning NO its reputation as a "double-edged sword."[52] Nitric oxide is formed by enzymes known as NO synthases (NOS) from L-arginine and molecular oxygen.[46] Like ET-1, it may be produced in endothelial cells and vascular smooth-muscle cells, depending on the circumstances. Also like ET-1, the genes responsible for its generation (the NOS genes) are highly conserved in evolution and display a high degree of homology between species.[5,52] The regulation of NOS, and hence NO production, is extremely complex.[48] and the very short biological half-life of NO causes its actions to be highly localized. The NOS are functionally classified into two types, constitutive and inducible. The constitutive NOS are present in unstimulated cells, Ca++ dependent, and subdivided into neuronal and endothelial types (after the cells in which they were first identified). The inducible NOS (iNOS) are distinguished by their absence in resting, unstimulated cells. They are generally Ca++ independent and their expression may be induced in many cell types, including endothelial and vascular smooth-muscle cells, under certain conditions, particularly exposure to cytokines (tumor necrosis factor-alpha, and gamma-interferon) and lipopolysaccharides.[51] There is recent molecular biological evidence that iNOS subtypes exist.[5] The exact role of the NOS and of NO in cerebrovasculature and ischemia is uncertain, and evidence exists for both protective[27,47,82] and deleterious[9,10,22,28] effects. Recent evidence indicates that the neuronal form of NOS may be deleterious in ischemic conditions, whereas the endothelial type may be protective by means of its vasodilatory effect; the development of relatively selective NOS inhibitors is needed to shed more light on these interactions.[9,80] Upregulation of gene expression of the NOS (both neuronal and inducible) has been demonstrated in models of brain ischemia[29,83] and suggests a pathogenic role on the one hand and a neuroprotective role on the other.[83] Furthermore, iNOS are known to be expressed in blood vessels following many forms of vascular injury.[48] This raises the question of whether they might be similarly expressed in cerebral blood vessels after vascular injury in the form of vasospasm, with its accompanying inflammatory response,[58] and if so, why this expression is apparently ineffective in promoting vasodilation independent of any brain effect, because NO has been demonstrated to increase regional cerebral blood flow.[47,82] It is noteworthy in this regard that the ET-1 gene is activated in response to exposure to interleukin-1.[81] 

RELATIONSHIP OF ET-1 AND NO TO CDCV

Particularly relevant features of these molecules include evolutionary conservation, high potency, short duration of action, complex physiology and regulation, local action, and physiological antagonism. These features suggest important biological roles including maintenance of normal vasomotor tone. It is tempting to speculate that CDCV represents a disturbance in the equilibrium for which these physiological antagonists are partially responsible.[7,14] To support this hypothesis, clinical features of aneurysmal SAH and CDCV may be invoked: CDCV is related to the volume of SAH; it is always associated with a delay in onset from the initial SAH of several days. Once the condition begins, it is progressive, chronic, and, eventually, self limited. The process appears to burn itself out after a period of days to 2 weeks in most cases. Hypotheses regarding the pathogenesis of CDCV attempt to accommodate these facts. Oxyhemoglobin lends itself to consideration as a pathogen in this context: as the major intracellular protein of the RBC it is liberated after lysis of this cell, and levels of oxyhemoglobin appear to peak in the cerebrospinal fluid (CSF) at 3 to 4 days.[4] Oxyhemoglobin has been demonstrated in vitro to activate the ET-1 gene and increase levels of ET-1 mRNA;[19,34,54] it is also eventually
enzymatically depleted in the CSF. Independent investigations have demonstrated gene activation in response to platelet products such as thrombin and transforming growth factor-β and to cytokines such as interleukin-1 as noted previously. These sorts of mechanisms appear to explain the clinical finding of increased ET-1 levels in CSF following SAH, which have been correlated with the incidence and time course of CDCV. Oxyhemoglobin is also known to inactivate NO by directly binding to its heme moiety. This phenomenon could explain the finding by some investigators that levels of soluble guanylate cyclase are inappropriately low in CDCV, thus the failure of intrinsic vasodilatory responses to compensate for arterial narrowing. The time course of clinical CDCV is consistent with the delayed and persistent rise in oxyhemoglobin levels that accompany RBC lysis. In short, the presence of oxyhemoglobin is capable of promoting vasoconstriction and inhibiting vasodilation by different but simultaneous mechanisms.

Hypotheses regarding the roles of oxyhemoglobin, ET-1, and NO in CDCV have been put to limited clinical and laboratory tests. Attempts to remove the influence of oxyhemoglobin have included subarachnoid lavage, thrombolysis, and administration of reducing agents to change the oxidation state of ferrous iron. The vasoconstrictor effects of ET-1 have been blunted by various means, but the most effective has been specific ET-A receptor blockade. Administration of NO in various forms has been used successfully to block and rapidly reverse ET-1- and SAH-induced CDCV in animal models. The future of these potential therapies is unknown because the biological roles of these molecules are far reaching, and the effect of removing their influence partially or entirely is unpredictable given the present state of knowledge. A genetic model in which the gene responsible for endothelial NOS has been eliminated, for example, demonstrates a certain resistance to the effects of cerebral ischemia but also demonstrates systemic hypertension. A similar gene knockout mouse with a deficient gene has been shown to manifest blood pressure and craniofacial anomalies. The administration of NO donors via an intravascular route has been discouraged because of effects on systemic hypotension, although recent work in which NO donors were administered intrathecally has met with some success in reversing ET-1-induced vasoconstriction.

CONCLUSIONS

Chronic delayed cerebral vasospasm, the same pathological entity described by Jackson in 1949 and alluded to in the context of aneurysm treatment by Alcock and Drake in 1965, has now emerged as the most important factor in morbidity and mortality rates associated with survival of aneurysmal SAH. Its mysteries have eluded and frustrated clinicians for more than three decades. The technical accomplishments of modern cerebrovascular surgery stand in stark contrast to this complication of aneurysmal SAH, which remains formidable and even deadly. Complex molecular and genetic interactions add to the burden of understanding this phenomenon. Preliminary work has begun to shed light on the molecular biological nature of CDCV implicating, among others, the genes responsible for ET-1 and NO. The intricate and variable physiological roles of these highly conserved biological molecules are slowly being examined in the context of SAH. This work has already begun to have an impact on the clinical research and treatment of CDCV. Continued work will further our understanding of these phenomena and will be ultimately rewarded with a specific and reliable treatment for CDCV.

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