Nitric oxide in focal cerebral ischemia, an experimental study

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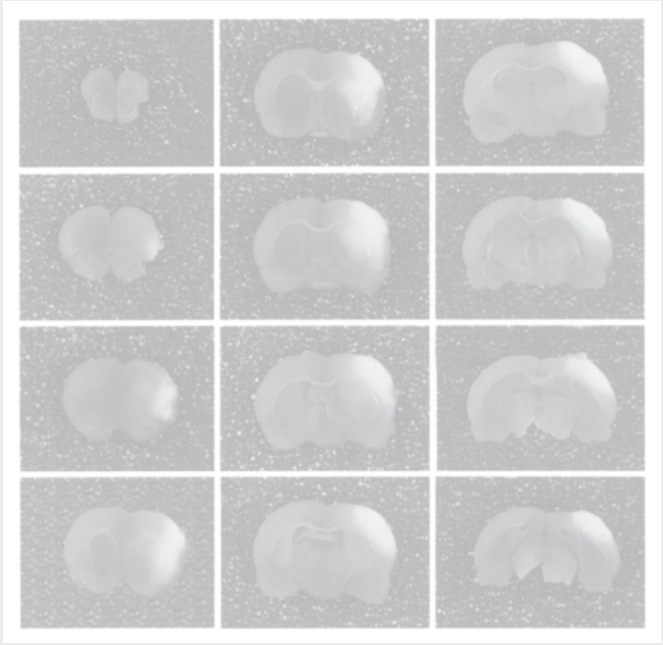
Chapter 7

Discussion



DISCUSSION

Model to clinic

The development of experimental models of focal cerebral ischemia has allowed for a better knowledge of its pathophysiology and for testing of therapeutic strategies. Numerous studies of neuroprotective compounds have shown reduction of infarct volumes in animal stroke models and in some cases promising phase II results while none have been proven efficacious on the basis of a positive phase III trial[26,30,45]. Many factors may have contributed to this phenomenon including: inadequate interpretation of pre-clincal data; underpowered phase III trials; patients are included that are unlikely to respond to the drugs being tested; the chosen primary endpoint may be inadequate to fully evaluate the drugs effect[24]. Phase III studies continue to be conducted despite limited pharmacokinetic data from animal and preliminary human studies[3,25]. Animal model deficiencies as well as inappropriate dosing and timing of therapy are also potential reasons for the failure of neuroprotective drugs in clinical efficacy trials[24]. Other important reasons for the negative neuroprotective trials probably are the lack of preclinical data to support the time window chosen for efficacy trials and the rapid movement to pivotal trials without in-depth understanding of side effect profiles and the subtypes of stroke patients that are most likely to benefit[24]. Stroke Therapy Academic Industry Roundtable (STAIR) meetings have focused on different aspects of the development and assessment of new neuroprotective stroke therapies. Robustness of the neuroprotective effects was found to be an important factor in determining which therapy should advance from preclinical to clinical development[1]. It was stated that the neuroprotective effects should be confirmed in at least two independent laboratories of which at least one should be independent of the sponsoring company[1]. Rather than moving directly to interventional studies in humans, the use of several appropriate animal models is encouraged[20]. The usage of at least two outcome measures was recommended to evaluate both functional and histological response[1,16]. Neurological deficits after experimental cerebral ischemia is sometimes difficult to detect in rodents[7]. Reports on the correlation between neurological deficits and stroke volumes have varied from no correlation[60] to significant correlations[51]. In clinical trials, however, functional recovery is the major end point[2].

No animal model can exactly mimic stroke in humans. The relevance to the human situation is essential before pharmacokinetic and time window issues can be resolved[67]. Comparison of positron emission tomography (PET) studies of stroke patients to a cat MCA occlusion model revealed great resemblance in the development of "misery perfused" penumbral tissue and its centrifugal conversion into necrosis,[31] which is important proof of relevance of this model. When embolic MCA occlusion in Sprague Dawley rats was directly compared to a suture MCA occlusion it was noted that there are important differences in acute ischemic lesion evolution[33], mimicking two different clinical

problems. The validity, complications and side effects were studied for three techniques of MCA occlusion by Gerriet et al. [29]. Subarachnoid hemorrhage was noted in some animals while in others the occlusion was inadequate with patent flow on MRA. In 7 of 37 cases, model failure was noted using MRA. This study provides us with important data on model failure. The age, gender species and strain of the laboratory animal used probably influence its relevance to stroke in humans[1]. Elderly animals had different response mechanisms, ischemic consequences and histological changes[52,53]. Frequently used young animal models may have limited efficacy in predicting clinical neuroprotective efficacy in a disease primarily affecting the elderly. Between different rat strains substantial differences in acute ischemic lesion evolution was demonstrated[6,48,61]. Gender specific differences in outcome were noted in different models,[11,62,63,65] raising the possibility that therapeutic interventions should be gender specific. In 1999 STAIR, rodent models of focal cerebral ischemia like the Tamura model[57] were recommended for the evaluation of putative neuroprotective drugs with careful dos-response and toxicology studies to enable future clinical trials[1]. To date new animal stroke models are being developed [32,36,63]. The development of models of focal cerebral ischemia must take into account known species differences and idiosyncrasies, underlying vascular disease processes, the nature of thrombotic processes, cellular reactivities, the presence of co-stimulation (e.g. inflammation), the characteristics of immunological and reporter molecules, the coincident use of other pharmacologic modifiers (e.g. anesthesia), and stress[20]. These elements are potential contributors to cerebral tissue injury and its assessment and may affect species differentially[20].

Stroke models have been a valuable instrument to study many facets of the pathophysiology of stroke. The transfer of these data to the clinical setting, however, has been mainly unsuccessful. This has made us realize that stroke models are a powerful tool in the quest to unravel the complex pathophysiology of stroke but care should be taken to extrapolate data to the clinical situation in humans. Stricter adherence to the recommendations based on previous experiences will hopefully prevent disappointing results from premature advancement to clinical trials.

Nitric Oxide in focal cerebral ischemia

An important and complex role for NO has been proposed in the pathophysiology of cerebral ischemia[17]. Whether overall NO is beneficial or detrimental seems to depend upon its dose timing and location[44]. NO is a short-lived, diffusible, reactive free radical gas that is synthesized from l-arginine through the NO synthase enzyme. Three isoforms were identified for this enzyme: type I neuronal or nNOS, type II inducible or iNOS and type III endothial or eNOS. While neuronal and endothial NOS were found in neurons en endothial cells respectively, inducible NOS was found in astrocytes. NO was found to

regulate vascular tone, platelet aggregation[49] and neurotransmission[55,18]. Under pathological circumstances it was also found to be an important mediator of N-methyl-d-aspartate (NMDA) mediated toxicity[19]. Direct measurements of NO in vivo are hindered by NO's short half life[4,59]. Uncoupling of constitutive NOS (endothelial and neuronal) leads to overproduction of superoxide (O_2^-) and peroxynitrite ($ONOO^-$), 2 potent oxidants and O_2^- and $ONOO^-$ which triggers the release of aggressive radicals[40]. Arginine analogue NO synthase inhibitors with selectivity for a specific iso-enzymes have been used to study the role of different sources of NO in cerebral ischemia[50,64]. Targeted gene disruptions of e- or nNOS isoforms are an alternative approach. Neuronal NOS knockout mice were found to be resistant to brain injury after focal cerebral ischemia[35] while e-NOS knockout mice developed larger infarcts[34]. This was consistent with studies using selective nNOS inhibitors[64,14] and data using nonselective NOS inhibitors that affect eNOS[14]. NO donor treatment mimicking endothelial NO[54] was found to protect brain tissue[15,66]. The endothelium plays a critical role in maintaining vascular tone by releasing nitric oxide (NO). Endothelium derived NO diffuses to smooth muscles, triggering their relaxation[5]. Modalities that upregulate eNOS expression and/or activity like HMG Co-A reductase inhibitors, steroid hormones, nutrients and physical activity were found to enhance cerebral blood flow and protect from ischemic stroke[23]. In animal models the protective effect of HMG Co-A reductase inhibitor simvastatin through eNOS activation was confirmed for adult animals if the statin was administered within 3-6 hours after ischemia[13]. Nitric oxide release from the endothelium of spontaneously hypertensive rats was found to be reduced when compared to controls[8]. The deficiency in NO concentration correlated positively with the increase of cerebral ischemia/reperfusion injury[21,38]. Racial differences in the predisposition to vascular diseases were explained by predispositions to endothelial dysfunction during ongoing vascular disturbances[37]. The clinical efficacy of third generation beta-adrenolytics like nebivolol was found to be through inhibition of endothelial dysfunction[42] and thus indirectly through release of NO[56]. Experimental evidence indicated that adventitial NO has an important role in the pathogenesis of cerebral vasospasm after SAH[47]. Markers of endothelial damage like von Willebrand factor were found to predict vasospasm[43,28,27]. A strong, graded and independent association was observed between blood concentrations of markers of endothelial activation (E selectin and Von Willebrand factor) and experimental ischemic stroke[12]. Nitric oxide synthase dysfunction is a therapeutic target in the treatment for delayed cerebral vasospasm after SAH[46,39]. A predisposition toward cerebral vasospasm may be related partially to genetic factors. In a study on 28 consecutive Fisher grade 3 patients with an aneurysmal subarachnoid hemorrhage DNA analysis revealed eNOS polymorphisms of the eNOS T 786C single nucleotide. Polymorphism correlated with the presence and severity of vasospasm[38].

In chapter 3 selective nNOS inhibition and eNOS augmentation reduced ischemic damage in experimental focal cerebral ischemia. Although very effective, selective nNOS inhibitors like 7-NI used in chapters 3 and 5 caused CNS depression[22], probably related to nNOS' physiological role. These side effects, but also the complex pharmacokinetics[10] and non water solubility make selective nNOS inhibitors like 7- NI less attractive. In contrast, NO donors have been used widely[9] since discovery of their vasodilatory effects. Currently focus is directed more towards the endothelium and eNOS. Better understanding of the role of NO from its various sources under physiologic and pathologic circumstances will hopefully lead to new therapeutic options.

The discovery of NO has led to a whole new field of research. The absence of an easy way to directly measure NO [41] has led to more indirect approaches yielding results which are inherently more difficult to interpret. Convincingly it has been demonstrated that inhibition of the neuronal isoform of NOS leads to smaller cerebral infarcts. The use of neuronal NOS inhibitors however, is limited by serious side effects that appear to be a direct result its physiological function. Exogenous NO (NO donor treatement), mimicking enhancement of endothelial NO, and l-arginine, the substrate of nitric oxide synthase and the main precursor of nitric oxide have similar effects[58]. Attention has shifted to eNOS which seem to play a pivotal role in the protective effects of statins and exercise in stroke and cardiovascular disease. The observed variability in ischemic complications after subarachnoid hemorrhage has been found to correlate with polymorphisms in e-NOS, confirming an important role for eNOS. Whether this will lead to therapeutic options remains to be seen. Further studies will have to clarify the effects of NO on platelet and endothelial function, but in this process, we need better techniques to measure or visualize NO. Nevertheless, NO will remain an important subject of study in future cerebrovascular research.

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