Structure-function analysis of neuroprotectants

In "The chemistry of neuroprotection", the author argues convincingly that there could be great benefit from a systematic and rigorously scientific study of the physical chemistry of putative neuroprotectants vis-à-vis their pharmacological effect. However, the first example used of the earliest thinking in this direction (which comes, not surprisingly via V. A. Negovskii, the father of resuscitation[1] medicine) is instructive as to some of the potential barriers standing in the way of this approach.

"It is not surprising that all the agents which are effective in shock carry a negative charge. This applies both to heparin, which possesses a very strong negative charge, and to hypertonic glucose solution. The same may be said about a substance now in wide use - dextran - which has small, negatively charged molecules, and also about the glucocorticoids 21, 17, and 11, which also have a negative charge." - Professor Laborit in: Acute problems in resuscitation and hypothermia; proceedings of a symposium on the application of deep hypothermia in terminal states, September 15-19, 1964. Edited by V. A. Negovskii.

In the intervening decades since Laborit wrote the words quoted above, supraphysiologic (high) steroids have not only failed to demonstrate benefit in cerebral resuscitation and shock, they have been found to be actively harmful in every well designed RCT undertaken to test their utility (a). This also extends to their lack of utility in trauma, spinal cord injury and sepsis. Similarly, the utility of heparin in treating the encephalopathy of the post-resuscitation syndrome, or improving survival after cardiac arrest has recently been called into question. Glucose, hypertonic or otherwise, was long ago demonstrated to markedly increase neurological injury if given immediately after reperfusion following cardiac arrest, and elevated blood levels of glucose, both pre- and post cardiac arrest have a strong negative correlation with both survival and neurological outcome.

Determining the seriously harmful effects of steroid administration in critical illness took decades. Despite the compelling evidence for their injurious effects, administration of large, supraphysiologic doses of steroids is still a practice both used and defended by some clinicians (albeit not ones who rely on evidence based criteria) and the use of glucose in shock, trauma and cardiac arrest took a nearly comparable period of time to discredit. These two examples are noteworthy because they comprised mainstays of therapy for most kinds of neuroinjury for decades, and they had compelling theoretical appeal, as well as many positive small clinical and animal research studies. Indeed, the debate continues to this day with controversy centred mostly on the use of low or "physiological replacement" doses of steroids in

[1] Resuscitation medicine is properly termed *reanimatology*, and is so-called in the non-English speaking world

critical illness. As the eminent pulmonologist and intensivist Neil Macintyre observed in 2005, “Patients die, but steroids never do.” This raises the twin problems of bad research (i.e., junk science) and statistically under powered or otherwise flawed studies. Combined, it has been estimated that these two types of defective studies comprise the bulk of published peer-reviewed scientific work.

High dose corticosteroid therapy for neuroinjury offers another complication in determining the therapeutic efficacy of any drug that merits consideration as a neuroprotectant (new or old). While there is no doubt that high-dose corticosteroids are ineffective and deleterious in the clinical setting, there is also little doubt that these agents *are* neuroprotective in the laboratory setting under certain conditions and for discrete subpopulations of neurons. The reasons for the failure of translational research in the case of corticosteroids are complex, but are mostly attributable to crucial differences between the laboratory and the real world of clinical medicine. In the case of corticosteroids these differences are most significantly:

a. Delay from time of insult to time of treatment; in the laboratory the timing of interventions is uniform and is typically much shorter than is the case in the clinic where delays in both presentation and treatment are both long and highly variable.
b. Heterogeneity of injury in humans compared to animals; animal models of neuroinjury are highly standardized (location, extent, mechanics) whereas human patients present with diverse injuries inflicted in many complex and often poorly understood ways.
c. Species differences; not only are there large genetic differences between humans and rodents in general, there are dramatic differences in the native ability of rodents to both resist and overcome infection in comparison to humans.
d. Demographics and comorbidities: laboratory animals are comparatively very uniform genetically, are typically young and healthy and of the same age, do not have comorbid conditions such as hypertension, diabetes, atherosclerosis, obesity or the diminished physiological capacity and repair and regenerative capacity increasingly present in humans over the age of 25.
e. Rodents aren’t people and do not interact with investigators in ways that facilitate straightforward determination of an adverse affect such loss of short term memory, or other cognitive deficits. It is now understood that the corticosteroids are toxic to the neurons of the hippocampus in both rodents and men. However, injury from this adverse effect is not only more evident in men than in mice (or rats for that matter), it is only men who are capable of complaining about it.

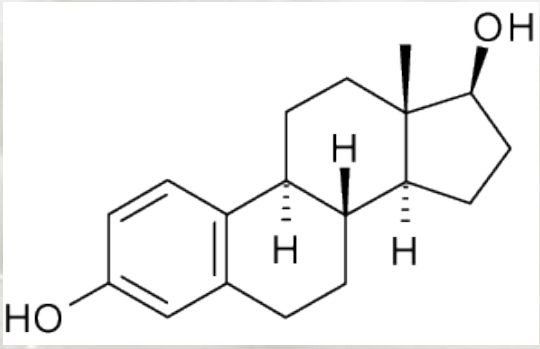
It is notable that all of these effects, with the possible exception of increased resistance to steroid-induced immunosuppression-mediated infection, obtain in the case of other translational models of drug development. The conclusion that corticosteroids are very likely neuroprotective in humans (in terms of the direct pharmacological effect on selected subpopulations of neurons in injured central nervous tissues under ideal conditions) is highly likely. However, the confounding

realities of the clinic and the genetic differences between men and rodents (the animals almost exclusively used in this type of research) mask this effect. This poses yet another serious challenge to investigators seeking to establish common moieties in prospective neuroprotective molecules.

Clinical trials of putative neuroprotective substances have been overwhelmingly negative. This has been the outcome despite often stellar results achieved in animal models; often in diverse species in studies conducted by multiple investigators in different institutions and sometimes in different countries; none of whom have any obvious relationship, let alone one that might raise the specter of conflict of interest. In the last 6 years alone, over 1000 experimental papers and over 400 clinical articles have appeared on this subject. What this suggests is that the same deficiencies seen in studies reported upon in rest of the peer-reviewed biomedical literature also apply to studies of pharmacological intervention in neuroprotection. An inevitable conclusion is that until the signal to noise ratio improves, attempts to draw general conclusions about the shared, essential properties of neuroprotective molecules will be difficult at best, and unreliable or misleading at worst.

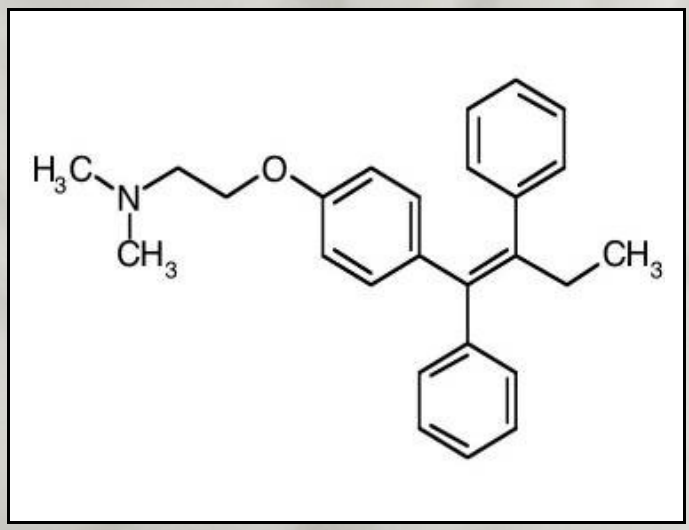
Perhaps a good place to start this kind of analysis is in an area where the molecular structure of the agent(s) is extraordinarily simple and the animal and clinical data are both robust and show good to fair agreement. Hypertonic sodium chloride solutions have demonstrated efficacy in providing both systemic (splanchnic) and cerebral protection in a broad class insults including hemorrhagic/hypovolemic shock, closed head injury and less robustly in stroke and global cerebral ischemia. Interestingly, other cation salts of chloride given at comparably high tonicity do not have this effect. Furthermore, animal as well as small human clinical studies have demonstrated isochloremic hypertonic solutions to be as effective as hypertonic sodium chloride at restoring microcirculatory flow and reversing metabolic acidosis in haemorrhagic shock without the potentially troublesome side-effect of raising the mean arterial pressure to levels where re-bleeding may occur in trauma or subarachnoid haemorrhage. A relative lack of effectiveness of the chloride salt of magnesium compared to the sulfate salt of this ion has also been noted. Understanding the mechanics of these paradoxes would seem to be a worthwhile and comparatively straightforward place to begin such structure-activity relationship analyses.

17β-Estradiol



Cerebroprotective drugs not infrequently possess a multiplicity of pharmacological effects that are known to be neuroprotective but that may be accomplished by very different and even indirect means in terms of their structure-function relationship. Some cerebroprotective molecules, such as the female hormone 17β-estradiol and the mixed estrogen antagonist-agonist tamoxifen share common physiochemical properties such as free radical scavenging, N-methyl-d-aspartate (NMDA) receptor inhibition, and modulation of volume regulated anion channels (VRAC); which play a role in ischemia-induced release of excitatory amino acids. There is considerable evidence that some of 17β-estradiol's neuroprotective effect is via signal transduction as well as its neurotrophic effects, even at doses below those necessary for its direct effects on reactive oxygen species production and its NMDA receptor inhibiting effects. While the structure of the molecules shares some important features, they are also structurally very different and the signal transduction and neurohormonal effects are almost certainly very different. Thus, these molecules also present a fascinating opportunity to probe structure-function relationships in neuropharmacology.

Tamoxifen



Finally, an admission, or perhaps a confession is order in ending this discussion. This author has been responsible for the application of at least one putative neuroprotective drug to cryopatients which ultimately proved ineffective in human clinical trials when administered during and after cardiopulmonary resuscitation (CPR). This drug, nimodipine, performed well in animal trials, but failed to show benefit in human trials, possibly as a result of its hypotension-inducing effect. Adequate mean arterial pressure (MAP) following resuscitation from cardiac arrest is essential to survival and a post arrest bout of hypertension has been demonstrated to provide substantial cerebral rescue in animal models of global cerebral ischemia. Reduction of MAP in cryopatients is a serious concern because achieving adequate perfusion pressure is problematic under the best of conditions. It is also worth noting that cryopatients have been given a variety of other ineffective neuroprotective drugs over the past 30 years, including the opiate agonist naloxone, the corticosteroid methylprednisolone and the iron chelating drug desferroxamine.

While these drugs, with the possible exception of nimodipine, are not likely to have been injurious (except perhaps to the pocketbook), their use raises important questions about when and how promising animal research should be translated to the setting of clinical cryonics. Unique among all other populations of human and animal patients, cryopatients have the opportunity to be treated with neuroprotective drugs that show great promise, absent the long delays of regulatory vetting, and independent of the economic pressure experienced by pharmaceutical companies to not only market drugs that are effective, but to market ones that are also profitable. The question thus becomes what criteria do we use in applying these drugs absent the extensive pre- and post marketing evaluation that obtains with approved ethical drugs? In essence the question we must ask and answer is “can we do better, much better in fact, than our colleagues in conventional critical care medicine?

(a) The one condition in which there is unequivocal benefit to supraphysiologic administration of steroids is meningococcal meningitis with substantial evidence also supporting a similar degree of efficacy in Typhoid and Pneumocystis carinii pneumonia..

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