Argon, a noble foe for subarachnoid hemorrhage

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1
Subarachnoid hemorrhage (SAH) is a serious health issue with a worldwide incidence of approximately 9 per 100,000 and a 6 month mortality of around 60% [1]. Those patients that survive are often severely impaired with associated long-term care and rehabilitation costs. Surgical management of SAH as a result of a cerebral aneurysm focuses on stopping bleeding through direct surgical clipping or endovascular repair (coil or glue embolization). Non-invasive management involves drugs to avoid vasospasm and maintain cerebral perfusion pressure, aimed at limiting the effects of early brain injury thought to underlie the majority of the disability burden in SAH survivors.

Argon is a member of the series of inert or “noble” gases with helium being the lightest and xenon the heaviest non-radioactive member. Despite having low chemical reactivity certain noble gases have biological activity; xenon is a general anesthetic at atmospheric pressure (MAC=0.63atm) while krypton and argon are anesthetics at elevated pressure (4.5atm and 15atm respectively), but are without measurable anesthetic effect at 1atm. Xenon has clearly identified pharmacological targets (e.g. NMDA receptors; TREK-1 channels, K-ATP channels) and has been demonstrated to be neuroprotective in a variety of models of ischemic & traumatic brain injury [2-6]. Argon has been less widely studied but has been shown to be neuroprotective in in vitro models of ischemic and traumatic brain injury [5, 7] and in vivo in models of ischemic injury [8-10]. Argon is more abundant and less expensive to produce than xenon and, if equally safe & effective as a neuroprotectant, may be advantageous.

The study by Höllig & colleagues [11] is the first reported study to evaluate the potential of argon as a treatment for SAH in a rodent model. Höllig et al used a rat model of SAH involving isolation of a common carotid artery and surgical insertion of a suture or wire into the internal carotid artery. The suture is advanced to the region of the branch of the middle cerebral artery / anterior cerebral artery (ACA). The filament is further advanced to rupture the ACA and is then withdrawn. This results in a severe and poorly controlled bleeding that resembles a ruptured cerebral aneurysm. Mortality is high in this rat SAH model, up to 40% in the first few days, which mirrors the clinical scenario.

The induction of SAH in this model involves surgery under general anesthesia, with monitoring of intracranial pressure and cerebral blood flow to confirm SAH. One hour after induction of SAH or sham surgery (insertion and removal of filament without vessel rupture), the rats breathed either 50% Ar:50% O2 or 50% N2:50% O2 through a facemask for a period of 1hr. The primary outcome measure was survival up to 6hr, 24hr and 72hr after SAH. Secondary outcomes were neurological scoring at 24hr, open field test at 24hr (a measure of locomotor function and exploratory behavior), brain tissue analysis for the stress-response proteins hypoxia-inducible factor (HIF1a) and heme-oxygenase (HO-1) at 6hr, 24hr and 72hr and hippocampal cell counting at 6hr, 24hr and 72hr.

The authors found survival was improved in the argon-treated groups, compared to nitrogen-treated groups at each time point. Multivariate statistical analysis indicated a significant reduction in risk of premature death of 20.6 % in the argon treated groups (p<0.05).

One potential confounder is the pooling of different time-points for the mortality analysis. The data shown in Figure 3 show that there are fewer deaths in the argon-treated groups compared to nitrogen-controls. This is striking at the two early time-points (6hr, 24hr) but less so at 72hr. However, because of the experimental design, each of the time-points, 6hr, 24hr and 72hr consists of a separate control and argon group (surviving animals are euthanized at each time-point). The reasons for this design are related to the requirement for post-mortem tissue samples at each time-point for the secondary outcomes. Unfortunately this reduces the power of the study to determine treatment-effect on survival, compared to the ideal scenario of two larger groups all surviving until 72hr.
With the current design it is impossible to tell whether argon-treatment improves survival up to 72hr or simply delays death within a 72hr period (at 72hr the number of deaths in each group only differs by 1 animal out of 9). This information is of high clinical relevance and could be provided by a study with larger groups of animals all of which are monitored for survival at each time-point, and ideally for longer survival times.

Another potential confounder of the current study is that it used two different methods of induction of SAH, namely the "classic" filament suture method and a "modified" wire filament method, which the authors [12] and others [13] have shown to have differing rates of mortality. The data for the two models were included in balanced proportions, and are combined in the figures and survival analysis. The authors argue that having different models of injury reflects the heterogeneity of clinical presentation. However, the introduction of this extra level of heterogeneity is at the expense of two advantages of (ideal) animal models which are reproducibility and controllability. Whether argon is effective in both milder and severe SAH is a question that has clinical relevance, and that could be answered by additional studies using both models with larger group sizes.

For the secondary outcomes, a decrease in the number of neurons in the dentate gyrus (but not other hippocampal regions) was observed in control SAH animals compared to sham surgery, which was abolished with argon-treatment. Argon-treatment elevated levels of HIF-1α and HO-1 at the 24hr time-point, but results were contradictory at other time-points. Unfortunately, this did not translate to functional outcome as no improvements were found in neurological scoring or open-field test of behavior in the argon-treated compared to control groups, but the authors report that bodyweight was significantly greater in the argon treated animals at each time point. The lack of a functional improvement in the argon-treated animals is disappointing, and may have been a driver in including the less severe SAH model in the study.

Animal models of human pathologies should ideally satisfy a number of (sometimes conflicting) criteria. There should be good replication of the pathology in the animal model, ideally the injury should be reproducible and controllable, and outcome measures used should be relevant and sufficiently sensitive to distinguish different injury severities. This is particularly challenging when both the human pathology and the animal model have a high early mortality, as is the case in SAH. When the objective is to evaluate the efficacy of an intervention in a pre-clinical model, the importance of reproducibility and sensitive outcome measures are amplified. If the injury is irreproducible or too severe or the outcome measure insensitive, then very large numbers of animals may be required to reveal an effect of the treatment. The inclusion of the "modified" less severe SAH model may have been an attempt to increase the sensitivity of the behavioral outcomes. It is unfortunate that the numbers of animals used in each model were apparently not sufficient to determine efficacy individually at different injury severities.

The authors should be commended for providing detailed information in supplementary material on the different injury models, censoring times, and exact times of death of each animal that are valuable and will be of great use in designing future studies. Translational research in the field of treatments for acute brain injury (notably ischemic stroke) is littered with promising animal results that failed to translate to humans. The reasons for this have been discussed at length in reviews (see eg [14]) and have led to calls for pre-clinical research to be treated similarly to clinical trials with moves for standardized pre-clinical protocols, registration of pre-clinical studies, duplication of findings at multiple institutions and publication of results whether positive or negative to avoid publication bias.

The question of whether argon is a potential treatment for SAH remains open. Nevertheless, this first study by Höllig and colleagues is another step in the search for a treatment for SAH.
References


