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Argon: a noble, but not inert, treatment for brain trauma?

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Traumatic brain injury (TBI) is an acquired neurological condition resulting from external mechanical forces and is a leading cause of morbidity and mortality worldwide.[1] The global incidence of TBI is estimated to be 60 million cases annually.[2] Although improvements in the clinical management of TBI have improved outcomes and reduced mortality, current treatments are largely supportive focussing on maintaining what are considered to be appropriate physiological levels of, for example, tissue oxygenation and ICP.[1,3]

Advances in the understanding of TBI pathophysiology have revealed a complex network of interacting cellular responses and biochemical pathways underlying the potentially preventable secondary injury that occurs after the mechanical insult.[4,5] However, clinically proven treatments for TBI specifically targeting neuronal injury and secondary injury pathways are currently lacking. Despite the apparent molecular complexity of the injurious processes underlying secondary injury, effective treatment could involve the use of a simple monatomic gas. Argon is a member of the series of noble gases that have low chemical reactivity and comprise group 18 of the periodic table, but they are neither inert nor biologically inactive. Argon, together with its larger neighbour krypton, has anaesthetic properties at elevated pressure, while xenon is an anaesthetic under normobaric conditions.[6] Argon has previously been evaluated in preclinical models of ischaemic stroke with both positive[7,8] and negative[9] findings. Both argon and xenon reduce injury development after TBI *in vitro*,[10–13] and xenon has been shown to reduce secondary injury, improve long-term locomotor function, prevent late-onset cognitive impairment and neuronal loss, and improve long-term survival after experimental TBI in mice.[14,15] Thus far there have been no reports of the efficacy of argon in an animal model of TBI. The study by Moro and colleagues[16] in the current issue of the *British Journal of Anaesthesia* represents a significant advance in this respect, with an evaluation of argon as a potential treatment for TBI in mice.

Moro and colleagues[16] used the highly reproducible controlled cortical impact model of blunt contusional TBI to investigate the efficacy of argon in mice after a severe injury. Spontaneously breathing male mice were treated with argon 70%:oxygen 30% for 24 h starting 10 min after brain injury. The authors used two measures of sensorimotor function: simple neuroassessment of asymmetric impairment (SNAP) and neuroscore. Argon treatment improved both the SNAP score and the neuroscore at 24 h and 7 days after injury, but not at 2, 3, or 6 weeks after injury. Motor impairment was reduced in the argon treatment group, assessed by counting the number of missteps as mice walk along a narrow beam 2 days after injury, but impairment was not assessed at later time points. Argon treatment also reduced memory impairment at 4 weeks after TBI, measured using the Barnes maze paradigm that assesses hippocampus-dependent spatial learning and memory by quantifying the time taken to find a hidden escape box after learning the maze during a series of training trials. These behavioural outcomes are clinically relevant as deficits in locomotor function and learning and memory are frequent clinical sequelae of TBI. However, the long-term persistence of the treatment effects is as yet unclear.

Another translationally relevant aspect of the study is the utilisation of MRI to quantify vasogenic oedema and white matter integrity. Diffusion weighted imaging at 3 days after injury revealed significant reduction in oedema in the argon-treated group. White matter damage at 5 weeks after TBI, assessed using diffusion tensor imaging, was reduced in the ipsilateral fimbria that connects the hippocampus with subcortical brain regions and in the corpus callosum in the argon-treated group. Although oedema volume is not routinely measured clinically, diffusion tensor imaging is used as a surrogate outcome measure for white matter injury in clinical trials.[17] At the cellular level Moro and colleagues[16] show that argon treatment reduced the number of pro-

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inflammatory microglia in the ipsilateral cortex, hippocampus, corpus callosum, and fimbria.

The strengths of the study are use of the well-characterised controlled cortical impact model, randomisation and blinding, relatively large group sizes for animal studies (n=20 in each group for behavioural tests up to 1 week after injury), clinically relevant behaviour outcomes, use of translationally relevant imaging modalities, and inclusion of supplementary data. Nevertheless, there are a number of caveats before firm conclusions on the efficacy of argon for TBI can be drawn. From a clinical translation perspective, perhaps the greatest limitation is the short time window of 10 min between injury and the start of argon treatment. The preclinical literature on acquired brain injury is littered with putative treatments that have failed to translate to human treatments.[18] There are many possible explanations for these failures, but a significant factor has been the failure to determine whether there is a clinically relevant time window for treatments. In some preclinical studies, treatments have been given before or during the injury thus potentially modulating the magnitude of initial insult in the treatment group. This is not the case here where treatment was given 10 min after trauma. Although there are specific circumstances where a treatment could be given within minutes of injury by first responders, there are likely to be limited opportunities for such early interventions. A more realistic clinically relevant time window would extend to a few hours (or at least the first hour) after injury, by which time a patient would have reached hospital. In the dynamic and rapidly evolving pathophysiology of TBI, treatments aimed at reducing or preventing secondary injury are likely to need to be started within hours.

As Moro and colleagues[16] acknowledge, further preclinical studies with argon are required to establish the time window within which efficacy is maintained. Another aspect is that the authors used a relatively high concentration of 70% argon. Although this makes sense for early preclinical studies on efficacy, in clinical TBI, oxygen concentrations in excess of 30% may well be required. It would therefore be essential to determine whether lower concentrations of argon (e.g. 50%) are equally effective, as noted by the authors.

The study should be commended for looking at outcomes up to 6 weeks after injury. This is a relatively long time for animal studies, and given the short lifespan of rodents, is equivalent to several months or a few years in humans. Given the clear association of TBI with increased risk of premature mortality and dementia in humans,[19,20] another area for further investigation would be to look at even longer-term outcomes. The authors' laboratory has been one of relatively few, along with our own, to study TBI in rodents up to 1 yr or more after injury,[15,21,22] and an investigation of argon's long-term efficacy would be very helpful.

An intriguing aspect of the study is that argon treatment improved hippocampus-dependent memory function in the Barnes maze test, but there was no difference in neuronal density in the ipsilateral hippocampus in the argon group, although there was a reduction in inflammatory microglia. The lack of a neuronal correlate of the functional outcome may seem strange, and microglia are known to modulate synaptic connectivity, however, it should be noted that the histopathology was at a different time point (7 days) than the Barnes maze test (4 weeks) and in a different cohort of animals. The authors focussed their attention on analysing the ipsilateral hemisphere and regions proximal to the severe impact site; it would be interesting to know whether there is neuronal loss, and possibly preservation with argon treatment, in the contralateral hemisphere. This information is of clinical relevance, given that in TBI patients there is the well-known coup-contrecoup phenomenon and that injury is often observed distant from the site of the primary injury.

In spite of these caveats, the work of Moro and colleagues[16] represents an important first step in the evaluation of the noble gas argon as a potential treatment for TBI. There are no clinically proven, specifically targeted neuroprotective treatments for TBI, and noble gases such as argon and xenon are attractive candidates. Attention has focussed on xenon, which is already approved for use as a general anaesthetic and has undergone a successful early clinical trial for ischaemic brain injury.[17] Xenon is effective in preventing short- and very long-term deficits after experimental TBI in animals.[14,15] However, the current work on argon for TBI, and other studies on argon for ischaemic brain injury,[7–9,11,23] suggest that this noble gas may also have promise as a treatment for acquired brain injuries. Argon is less expensive than xenon, and if it is equally as effective as xenon, this could favour its use in the absence of closed rebreathing circuits. As argon and xenon appear to act via different mechanisms,[13,23] there is the possibility that combinations of these two noble gases could have a synergistic effect. However, caution should be exercised before human trials of argon, because many treatments that have shown promise in brain injury in animals have failed to translate to humans.[18] One of the features of TBI that makes human trials challenging is that TBI is a very heterogenous condition both in severity of injury and in age of patients. In order to avoid the failures of the past, rigorous preclinical data packages should be assembled including determining the therapeutic time window, concentration response, efficacy in different injury severities (in both female and male animals of different ages), efficacy in other species, and a clear mechanism of action. This interesting first study by Moro and colleagues[16] is an important first step that should prompt additional research into argon as a potential novel treatment for TBI.

Authors' contributions

Both authors made substantial contributions to the article, revised the article content, approved the final version, and agreed to be accountable for all aspects of the work: both authors.

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Declarations of interest

The authors declare that they have no conflicts of interest.

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