Assessment of the neuroprotective efficacy of poly-arginine-18 (R18) peptides in a pre-clinical model of perinatal hypoxic-ischaemic encephalopathy (HIE)

Adam Edwards
The University of Notre Dame Australia
Chapter 1

General Introduction
1.1 INTRODUCTION

Perinatal hypoxic-ischaemic encephalopathy (HIE; also referred to as perinatal asphyxia) is a devastating condition, which affects the brain of term (> 36 weeks gestation) and pre-term (≤ 36 weeks gestation) infants. Brain injury associated with HIE is a leading cause of mortality among infants, while survivors are often left with lifelong disabilities such as cerebral palsy, epilepsy, intellectual disability and autism spectrum disorders (Kolevzon, Gross, & Reichenberg, 2007; O’Shea, 2008; Perlman, 1997; Pisani et al., 2009). There are currently no clinically available pharmacological neuroprotective treatments to reduce brain injury following HIE. For this reason, there is an urgent need for the development of a safe and widely applicable neuroprotective agent for infants who suffer HIE. Current clinical interventions to minimise HIE are limited to the use of hypothermia in term infants. However, the availability of a neuroprotective agent that can be administered soon after hypoxia-ischaemia (HI), to both pre-term and term infants, either in combination with hypothermia or alone (e.g. when hypothermia therapy is not an available treatment option), would provide the best opportunity to preserve brain tissue and thereby reduce mortality, improve patient quality of life and lessen the social and economic impact of this devastating condition.

Recent studies in A/Prof Meloni’s laboratory have demonstrated that cationic arginine-rich peptides and poly-arginine peptides (hereafter referred to as CARPs, unless otherwise stated) have potent neuroprotective properties in in vitro and animal injury models that mimic the effects of brain ischaemia (Meloni, Brookes, et al., 2015; Meloni et al., 2014, 2017; Meloni, Milani, et al., 2015; Milani et al., 2018, 2017; Milani, Clark, et al., 2016; Milani, Knuckey, Anderton, Cross, & Meloni, 2016). To
determine the neuroprotective potential of CARPs as a neuroprotective therapy for HIE, initial pre-clinical studies using in vitro and a small animal model of HIE are required. Consequently, the focus of this thesis was to examine the efficacy of the poly-arginine peptide R18 in HIE by using an in vitro neuronal cell excitotoxicity model and in an in vivo model of HIE in the P7 rat.

1.2 PERINATAL HYPOXIC-ISCHAEMIC ENCEPHALOPATHY (HIE)

1.2.1 Epidemiology

Perinatal HIE remains a leading cause of infant mortality and morbidity, accounting for 23% of infant mortality worldwide (Lawn, Shibuya, & Stein, 2005), with an incidence in developed countries of 2 – 6 per 1,000 live term births and 7 per 1,000 live pre-term births (Chalak et al., 2012; de Haan et al., 2006; Hankins & Speer, 2003; Wall et al., 2010). In developing countries, the incidence drastically increases to 30 – 100 per 1,000 live births overall (Simiyu, Mchaile, Katsongeri, Philemon, & Msuya, 2017). Of infants surviving HIE, 10% demonstrate persistent motor abnormalities and 50% demonstrate sensory and/or cognitive abnormalities (Hack et al., 1992; Lee et al., 2013). The frequency of motor and cognitive disorders associated with HIE has remained stable since the 1990s (Vincer et al., 2006; Wilson-Costello et al., 2007).

1.2.2 Aetiology

The aetiology associated with HIE represents the complexity of this neurological condition. Risk factors associated with HIE are summarised in Table 1.1 and include
intrapartum insults (e.g. nuchal cord, umbilical cord knotting, umbilical haemorrhage, placental abruption, pre-eclampsia and shoulder dystocia) and demographic factors (e.g. nulliparity and gestational age of foetus).

Table 1.1 Risk factors associated with HIE

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuchal cord</td>
<td>Nuchal cord increases the risk of HIE due to the reduction of cerebral blood flow through the compression of arterial and venous flow in the fetal neck</td>
<td>Peesay, 2017</td>
</tr>
<tr>
<td>Umbilical cord knotting</td>
<td>Umbilical cord knotting increases the risk of HIE due to the reduction in perfusion of blood to the foetus via the impairment of arterial and venous flow in the umbilical cord.</td>
<td>Peesay, 2017</td>
</tr>
<tr>
<td>Umbilical haemorrhage</td>
<td>Umbilical haemorrhage increases the risk of HIE due to the reduction in perfusion of blood through the foetus</td>
<td>Scutiero et al., 2018</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>Detachment of the placenta from the uterus increases the risk of HIE due to the reduction in the perfusion of blood to the placenta and foetus</td>
<td>Downes, Grantz, &amp; Shenassa, 2017</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>Disorders of maternal hypertension increase the risk of HIE due to the development of placental lesions, leading to a reduction in the perfusion of blood to the foetus</td>
<td>Martinello, Hart, Yap, Mitra, &amp; Robertson, 2017</td>
</tr>
<tr>
<td>Shoulder dystocia</td>
<td>Shoulder dystocia is known to increase the risk of HIE</td>
<td>Politi, D’Emidio, Cignini, Giorlandino, Giorlandino, 2010</td>
</tr>
<tr>
<td>Maternal infection</td>
<td>Maternal infections are known to increase the risk of HIE</td>
<td>Jenster et al., 2014</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>An increased risk of HIE is associated with first time pregnancies</td>
<td>Liljestrom, Wikstrom, Agren, &amp; Jonsson, 2018</td>
</tr>
<tr>
<td>Gestational age</td>
<td>An increased risk of HIE is associated with lower gestational age</td>
<td>(Ong et al., 2015)</td>
</tr>
</tbody>
</table>
1.2.3 Clinical aspects

The clinical presentation of symptoms following HIE is complex, with no early definitive diagnostic modality available to the clinician. Every infant born at 1 and 5 minutes of age is subjected to an Apgar assessment (inclusion criteria for therapeutic hypothermia, which is discussed below), which includes the assignment of a numerical score from 0 - 2 following examination of each of an infant’s appearance, pulse, grimace, activity and respiration; with a low score indicating potential infant demise. If HIE is suspected within the first hours of birth, a neurological examination of the infant is performed and scored using the Sarnat grading scale; 1 = mild HIE, 2 = moderate HIE and 3 = severe HIE (see Table 1.2). A diagnosis of HIE occurs through the combination of Apgar and Sarnat scores, and when possible magnetic resonance imaging and amplitude integrated/conventional electroencephalography. Generally, between the age of 18 – 24 months, confirmatory diagnostic tests are performed for infants who have suffered HIE. The Bayley Scales of Infant and Toddler Development assessment is a common confirmatory diagnostic test examining a child’s cognitive score, language score, motor score, cognitive delay, language delay and motor delay. Often it is not until later in life can an individual be definitively diagnosed with neurological sequelae associated with HIE (e.g. cerebral palsy, epilepsy, intellectual disability and autism spectrum disorders) (Driscoll, Felice, Kenny, Boylan, & O’Keeffe, 2018; Glass et al., 2009; Perlman, 1997; Robertson & Finer, 1993).
Table 1.2 Sarnat staging for diagnosis of HIE severity

<table>
<thead>
<tr>
<th>Severity</th>
<th>Stage 1 (Mild)</th>
<th>Stage 2 (Moderate)</th>
<th>Stage 3 (Severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of consciousness</td>
<td>Hyperalert</td>
<td>Lethargic or</td>
<td>Stupor or coma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>obtuned</td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>Normal</td>
<td>Decreased</td>
<td>Absent</td>
</tr>
<tr>
<td>Muscle tone</td>
<td>Normal</td>
<td>Mild hypertonia</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Posture</td>
<td>Mild distal flexion</td>
<td>Strong distal flexion</td>
<td>Intermittent decerebration</td>
</tr>
<tr>
<td>Stretch reflexes</td>
<td>Overactive</td>
<td>Overactive</td>
<td>Decreased or</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>absent</td>
</tr>
<tr>
<td>Suck</td>
<td>Weak</td>
<td>Weak or absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Moro (startle)</td>
<td>Strong</td>
<td>Weak</td>
<td>Absent</td>
</tr>
<tr>
<td>Tonic neck</td>
<td>Slight</td>
<td>Strong</td>
<td>Absent</td>
</tr>
<tr>
<td>Pupils</td>
<td>Mydriasis</td>
<td>Miosis</td>
<td>Variable</td>
</tr>
<tr>
<td>Heart rate</td>
<td>Tachycardia</td>
<td>Bradycardia</td>
<td>Variable</td>
</tr>
<tr>
<td>Seizures</td>
<td>None</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
</tbody>
</table>

1.3 PATHOPHYSIOLOGY

HIE occurs following an impediment of oxygenated cerebral blood flow (CBF), resulting in a pathophysiological cascade eventuating in programmed cell death. The fetal brain is particularly sensitive to a HI environment, due to the requirement of a constant supply of energy in the form of adenosine triphosphate (ATP). When this energy supply is interrupted, excitotoxicity occurs through the uncontrolled release of excitatory neurotransmitters such as glutamic acid; marking the beginning of the ischaemic cascade (Johnston, 1995). An in-depth assessment of pathophysiological cascades following HI is presented below and in Chapter 2.

1.3.1 Necrosis

Cellular necrosis is the primary cell death pathway initiated following severe HI (Northington, Chavez-Valdez, & Martin, 2011) and is defined by cellular swelling, karyolysis and lysis. Necrosis occurs following the failure of homeostasis due to an
excitotoxic influx of calcium and sodium, resulting in mitochondrial dysfunction and reactive oxygen species (ROS) production.

1.3.2 Apoptosis

Cellular apoptosis is the primary cell death pathway initiated following moderate HI (Northington et al., 2011) and is defined by cellular shrinkage, fragmentation into apoptotic bodies and rapid phagocytosis. Multiple apoptotic pathways have been demonstrated to be involved in HI, generally, apoptosis is ATP- and caspase-dependent. Following ATP depletion, calcium influx and the activation of calcium-dependent caspases, several pro-apoptotic proteins are released into the cytosol.

1.3.3 Excitotoxicity

Following prolonged HI, cellular homeostasis is disrupted and results in the failure of the cell (e.g. neurons, glia and glial precursor cells) to maintain ionic gradients, resulting in uncontrolled cellular depolarisation and release of glutamic acid into the synaptic cleft (Olney, Price, Samson, & Labruyere, 1986). High extracellular levels of glutamic acid cause cellular excitotoxicity, producing cytotoxic cellular influx of calcium ions. Increased intracellular levels of calcium activates calcium-dependent proteases, ROS production, cytotoxic oedema, mitochondrial dysfunction and the stimulation of pro-cell death pathways (Arai, Vanderklish, Kessler, Lee, & Lynch, 1991; Castilho, Ward, & Nicholls, 1999; O’Hare et al., 2005; Stout, Raphael, Kanterewicz, Klann, & Reynolds, 1998; Zhang, Dawson, Dawson, & Snyder, 1994).
1.3.4 Oxidative stress

As the developing brain is comprised of high levels of unsaturated fatty acids which are susceptible to lipid peroxidation, metals catalysing free radical reactions and low concentrations of antioxidants, the developing brain is highly susceptible to oxidative stress (Ikonomidou & Kaindl, 2011). Following HI, resident microglia produce excess inflammatory cytokines (IL-1β, TNF-α, etc.), glutamic acid, nitric oxide and ROS (Kaur, Rathnasamy, & Ling, 2013; Lai et al., 2017). Rapid increases in these levels of cytokines within cerebral tissue induces apoptosis, inhibits neurogenesis and contributes to immune cell migration to the site of HI injury.

1.3.5 Mitochondrial dysfunction

In cells affected by HI, several processes result in the activation of pro-apoptotic Bel-2 proteins (e.g. Bid, Bax, etc.), resulting in mitochondrial membrane permeability transition (MPT) (Bernardi et al., 2006; Wang et al., 2009). In addition, oxidative stress caused by ROS plays a critical role in mitochondrial dysfunction including ischaemic starvation, reperfusion-induced hyper activation and delayed neuronal death (Sanderson, Reynolds, Kumar, Przyklenk, & Hüttemann, 2013), prompting MPT and mitochondrial release of cytochrome-c. Cytosolic cytochrome-c, along with deoxyadenosine triphosphate, interact with apoptotic protease activating factor-1 (Apaf-1) to form the apoptosome, contributing to pro-apoptotic cell death via the activation of pro-caspase-9 (Thornton et al., 2012). Further alterations to mitochondria, such as mitochondrial outer membrane permeabilisation, results in the release of other pro-apoptotic factors, including apoptosis-inducing factor, endonuclease-G and second mitochondria-derived activator of caspases.
(smac)/DIABLO (Blomgren & Hagberg, 2006; Zhu et al., 2007), all of which mediate chromatin fragmentation and increase the activation of pro-apoptotic caspases, leading to cell death.

1.3.6 Inflammation

Following HI, resident microglia become activated in the cerebral parenchyma and develop macrophage-like attributes (e.g. phagocytic properties, cytokine production, matrix metalloproteinase release and antigen presentation) (Iadecola & Anrather, 2011). Specifically, the release of matrix metalloproteinases compromises blood brain barrier integrity, promoting the invasion of peripheral leukocytes into the cerebral parenchyma; exacerbating cerebral injury (Kaur, Sivakumar, Yip, & Ling, 2009). Like macrophages, astrocytes also become activated due to the release of pro-inflammatory cytokines, and ROS. Activated astrocytes subsequently release pro-inflammatory cytokines [interleukin (IL)-6, tumour necrosis factor (TNF)α, IL1α/β and interferon (INF)γ]; further exacerbating pro-cell death pathways (e.g. apoptosis) (Liu & Mccullough, 2013; Tuttolomondo, Di Raimondo, di Sciacca, Pinto, & Licata, 2008).

1.4 CURRENT ACUTE CLINICAL NEUROPROTECTIVE TREATMENT

Hypothermia

The use of hypothermia for the clinical treatment of HIE is presented in Chapter 2. Briefly, moderate hypothermia (33.5°C for 72 h) applied within 6 hours of birth improves the survival and neurodevelopmental outcomes in infants suffering from
moderate and severe HIE. While hypothermia is considered standard care in the treatment of infants with HIE and is well tolerated in term infants (Azzopardi et al., 2009; Gluckman et al., 2005; Jacobs et al., 2011; Shankaran et al., 2005), it does not provide complete protection. Of those term infants who have moderate or severe encephalopathy, therapeutic hypothermia was shown to decrease mortality from 40% to 28%, while in surviving infants, neurological morbidity was reduced from 31% to 19% of infants receiving hypothermia (Jacobs et al., 2013). The use of hypothermia is widely associated with increased risks of adverse events such as bradycardia and thrombocytopenia (Jacobs et al., 2013); although it is widely accepted that the risk of adverse events is outweighed by the neuroprotective outcomes demonstrated by a significant reduction in mortality or major neurodevelopmental disability 18 months after HIE (Jacobs et al., 2013).

Importantly, whilst hypothermia is safe in pre-term infants suffering necrotising enterocolitis (Hall et al., 2010), it has not been adequately evaluated for use in pre-term infants suffering HIE. Information regarding the safety and efficacy of hypothermia to treat pre-term infants suffering HIE is lacking, although information may be available in the near future (NCT1793129). In one study, hypothermia in pre-term infants was associated with higher mortality and increased clinical complications when compared to term infants, although the study did not include a normothermic pre-term control group (Rao et al., 2017).
1.5 OTHER CLINICAL NEUROPROTECTIVE APPROACHES UNDER CONSIDERATION

The following outlines neuroprotective approaches to treat HIE, that are currently, or have been assessed in human clinical trials.

1.5.1 Xenon

The noble gas xenon is an approved anaesthetic drug (Stoppe et al., 2013) with organoprotective and neuroprotective properties when administered alone or in combination with hypothermia in pre-clinical models of HIE (Banks, Franks, & Dickinson, 2010; David et al., 2003; Faulkner et al., 2011; Hobbs et al., 2008; Thoresen, Hobbs, Wood, Chakkarapani, & Dingley, 2009). Whilst the mechanism of action of xenon neuroprotection has not been elucidated, xenon is known to prevent N-methyl-D-aspartic acid (NMDA) receptor over activation via competitive inhibition at the glycine site of the NMDA receptor (Dickinson et al., 2007).

Two clinical studies have assessed the safety of xenon in term infants with HIE and treated with hypothermia (33.5°C for 72 h). In one study, treatment with xenon (50% for 18 h; inhaled) and hypothermia was demonstrated to be safe (Dingley et al., 2014; ISRCTN75602528). The second study also demonstrated that xenon (30% for 24 h; inhaled) and hypothermia treatment was safe (Azzopardi et al., 2016; NCT00934700 and ISRCTN08886155), but concluded that adjunct xenon therapy is unlikely to improve the neuroprotective effect of hypothermia.
1.5.2 Topiramate

Topiramate is an anti-epileptic drug approved for partial and generalised seizures in adults and children. It has neuroprotective properties in pre-clinical models of HIE when administered alone or in combination with hypothermia (Cowell, Xu, Galasso, & Silverstein, 2002). Topiramate has been demonstrated to confer neuroprotection through the reduction in sodium- and calcium-dependent action potentials (DeLorenzo, Sombati, & Coulter, 2000), enhancement of γ-aminobutyric acid-mediated neuronal chloride influx (White, Brown, Woodhead, Skeen, & Wolf, 1997) and the inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate type glutamic acid receptors (Koh & Jensen, 2001).

One clinical study has assessed the safety of topiramate in term infants with HIE and treated with hypothermia (33.5°C for 72 h) (Filippi et al., 2010; NCT01241019). Topiramate administration (10mg/kg once daily for 3 days; orally) was demonstrated to be safe; although hypothermia did slow the rate of clearance of topiramate from the body. A second clinical study has been completed, but the results have not been published to date (NCT01765218).

1.5.3 Pre-term therapeutic hypothermia

One clinical study has assessed the safety of hypothermia (33.5°C for 72 h) in pre-term neonates (34 – 35 weeks gestation) suffering HIE (Rao et al., 2017; NCT00620711). The study demonstrated that the use of hypothermia was feasible, but cautioned its use for future application due to the increased risk of mortality and side effects. Additional, clinical studies are currently recruiting patients for the investigation of safety and efficacy of hypothermia in pre-term infants.
(NCT01793129; 33.5°C for 72 h; 33 – 35 weeks gestation; expected completion October 2022 and NCT02676063; 33.5°C for 72 h; ≥ 34 weeks gestation; expected completion February 2021).

1.5.4 Antenatal magnesium sulfate (MgSO₄)

Magnesium is a non-competitive NMDA receptor antagonist and following HI-mediated excitotoxicity, magnesium is thought to prevent excitotoxic neuronal calcium influx (Kang et al., 2011) as well as reduce pro-inflammatory cytokines IL-6 and TNFα (Aryana, Rajaei, Bagheri, Karimi, & Dabbagh, 2014). For decades, MgSO₄ has been administered antenatally for the prevention of seizures in women with pre-eclampsia and eclampsia. Through antenatal administration, foetuses are passively exposed to MgSO₄; with fetal MgSO₄ levels almost half of the maternal blood levels (Sherwin et al., 2014).

Five clinical studies have assessed the safety and/or efficacy of MgSO₄ in pre-term infants suffering HIE when administered to the mother in the antepartum period. Three studies demonstrated that MgSO₄ administration 4g within 24 h of birth (Crowther et al., 2003; ACTRN12606000252516; Marret et al., 2006) or 4g bolus followed by 1 g/h for 24 h was safe, but did not appear to improve infant outcomes (The Magpie Trial Collective Group, 2002; ISRCTN86938761). One study (Rouse et al., 2008; NCT00014989), demonstrated MgSO₄ (6g bolus followed by 2g/h maintenance for 12 h) was safe and reduced the rates of cerebral palsy at 1 year of age. Another study (Mittendorf et al., 1997) was prematurely terminated due to high mortality rates in
infants born to mothers administrated MgSO₄ (4 g bolus, then 2 – 3 g/h infusion). A clinical study is currently recruiting patients (ACTRN12611000491965; 4g MgSO₄ within 24 h of birth).

1.5.5 Erythropoietin (EPO) and darbepoetin adjuvant to hypothermia

EPO is commonly used clinically for its erythropoietic properties to treat anaemia. Recombinant human EPO has been examined as a potential neuroprotective agent both in vitro and animal studies of HIE. Current dose regimens to elicit erythropoiesis are administered subcutaneously at 400 IU/kg, three times a week, or intravenously at 200 IU/kg, daily (Robertson et al., 2012). EPO is known to increase neural stem cell proliferation (Zhang et al., 2017) and encourage cerebrovascular remodelling (Keogh, Yu, & Wei, 2007). EPO has also been demonstrated to be neuroprotective in animal models of ischaemic stroke, traumatic brain injury, chronic hypoxia and HIE as well as improve neurodevelopmental outcomes in pre-term infants (discussed below). Despite EPO’s neuroprotective properties, an appropriate clinical therapeutic dose and confirmation of efficacy in HIE has not yet been ascertained. Assessment of EPO in rodent models of HIE has demonstrated that doses ranging from 1,000 to 5,000 IU/kg are neuroprotective (Gonzalez et al., 2009; Chung, Kong, Goldberg, Stowe, & Raman, 2015; Fan, van Bel, van der Kooij, Heijnen, & Groenendaal, 2013).

Clinical studies have assessed the safety of EPO and darbepoetin (a modified form of EPO), in term infants suffering HIE and treated with hypothermia (33.5°C for 72 h). These studies demonstrated that EPO or darbepoetin in combination with hypothermia was safe (Wu et al., 2012; NCT00719407; Baserga et al., 2015; NCT01471015). A
clinical study further assessing the efficacy of EPO with hypothermia in term infants is currently recruiting patients (NCT03079167; estimated completion December 2020).

1.6 CARPS AND NEUROPROTECTION

As previously outlined, this project aims to extend the previous findings that initially identified that CARPs, including poly-arginine peptides, possess intrinsic neuroprotective properties. Neuroprotective CARPs typically range in size from 6 – 30 amino acids and are cationic due to the presence of arginine, lysine and to a lesser extent histidine residues. CARPs have the capacity to cross the plasma membrane and enter cells, and for this reason they are also referred to as cell penetrating peptides (CPPs). CARPs can also cross the blood brain barrier and enter the brain. Due to their membrane traversing properties, CARPs (i.e. cationic CPPs) have been extensively used for the intracellular delivery of agents in experimental in vitro and animal studies (Meloni, Milani, et al., 2015), as well as for the delivery of agents into the brain. A review summarising the application of CARPs as a neuroprotective therapeutic treatment for HIE is presented in Chapter 2.

1.7 AIMS OF THE THESIS

The overall goal of this thesis is to examine the neuroprotective efficacy of CARPs, namely the poly-arginine peptide R18, in a rat model of HIE. In order to accomplish this goal, a P7 rat model of HIE was established and characterised. Using different treatment regimens (e.g. l- and d-enantiomer R18, different peptide doses and
administration time points post-HI) the HIE model was subsequently used to examine the efficacy of R18 to reduce brain injury and improve functional outcomes.

Specific aims of the project are:

**Aim 1:** To establish and characterise a more reliable and reproducible P7 rat model of HIE.

**Hypothesis:** Occlusion of the common and external carotid arteries (as opposed to occlusion of the common carotid artery only), followed by transient hypoxia will generate a more consistent brain lesion.

**Aim 2:** To examine the dose response neuroprotective efficacy of R18 (L- and D-enantiomers; R18 and R18D, respectively) using the newly established P7 rat model of HI (as described in Aim 1).

**Hypothesis:** R18 peptides are neuroprotective following HIE.

**Aim 3:** To examine the therapeutic time window and dose responsiveness of R18 (as identified in Aim 2) using the P7 rat model of HIE.

**Hypothesis:** R18 will be effective when administered several hours after the commencement of hypoxia.
1.8 REFERENCES


Dingley, J., Tooley, J., Liu, X., Scull-Brown, E., Elstad, M., Chakkarapani, E., …


Northington, F. J., Chavez-Valdez, R., & Martin, L. J. (2011). Neuronal cell death in


Sanderson, T. H., Reynolds, C. A., Kumar, R., Przyklenk, K., & Hüttemann, M.


observational study. *Medical Gas Research*, 3(1), 12.
https://doi.org/10.1186/2045-9912-3-12


