Microglia provide unexplored avenues to attenuate cognitive decline following isoflurane exposure in susceptible young and elderly patients: a critical review of TREM2-DAP12 function and therapeutic potential

IR Niesman1,2*, NM Carter2

Abstract

Introduction

Microglia are the principle mediators of neuroinflammation, as defined by increased expression of pro-inflammatory cytokines such as TNF-a, IL-1b and IL-6. Two patient populations, young and aged, are at significant risk for behavioural and cognitive impairments after isoflurane exposure. Evidence indicates that neuroinflammation may be one of the causative events leading to neuronal and synaptic loss. The aim of this review was to discuss how microglia provide unexplored avenues to attenuate cognitive decline following isoflurane exposure in susceptible young and elderly patients.

Conclusion

Isoflurane exposure affects the actin cytoskeleton in neurons and astrocytes, but cytoskeletal effects from isoflurane exposure have not been well studied in microglia. TREM2 expression, a known inflammatory and cytoskeletal regulating protein, is reduced in young and aged populations leading to depression of microglia phagocytosis and increased inflammation. Therefore, modulation of upstream or downstream TREM2-mediated signalling may be a route to attenuate cognitive impairments in both populations.

* Corresponding Author
E-mail: iniesman@ucsd.edu

1 Department of Anesthesiology, University of California-San Diego, 3350 La Jolla Village Drive, San Diego, CA 92161, USA
2 Department of Pharmacy, Health & Wellbeing, University of Sunderland, Sunderland SR1 3SD, UK

Introduction

Learning disabilities and behavioural issues in children and post-operative cognitive decline (POCD) in elderly patients after anaesthetic exposure pose a significant health issue adding extra pressure to often already overburdened school systems and long-term care facilities. A major risk factor for childhood development of testable cognitive impairment is multiple exposures of inhaled anaesthetics. Wilder et al. have defined a specific subpopulation of children at particular risk for long-term learning disabilities: children below the age of four who incur at least three surgical exposures1. In the elderly, POCD aetiology may involve multiple factors, such as nutritional states and metabolic states or the type of surgical procedure2. A majority of potential therapies or interventions currently being explored are aimed at attenuating neuronal apoptosis and synaptic loss or increasing neurotrophic survival factors such as the brain derived neurotrophic factor (BDNF)3. A recent review of 25 randomized clinical trials of neuroprotective pharmacological compounds including: lidocaine, thiopental, S(+) ketamine, propofol, nimodipine, GM1 ganglioside, lepianfatin, glutamate/aspartate, Xenon, remacemide, atorvastatin and magnesium sulphate, found all but three were ineffective at preventing POCD when compared to controls. Lidocaine, S(+) ketamine and magnesium sulphate had inconclusive results4. The inert noble gas Xenon has been proposed as a prophylactic treatment for POCD exposure during mouse surgeries. Pre-treatment of 70% Xenon for 20 minutes followed by 1.8% ISO during surgery reduced serum IL-1β levels but mice cognitive abilities were only tested post surgery P1.5. A novel approach to preventing POCD through activation of autophagic pathways with rapamycin to inhibit mTOR has been proposed6. But this approach is limited to use in an aged population, as increased autophagy is associated with increased neuronal apoptosis after ischemic injury in neonatal rat experiments7.

In this critical review, we propose a more focused therapeutic target to explore. Microglia, required to resolve neuroinflammatory states and modulation to a phagocytic phenotype without inducing neuroinflammation, could provide neuroprotection. The expression of triggering receptor expressed in myeloid cells 2 (TREM2) is associated with decreased phagocytic and increased inflammation. This reduction of testable cognitive impairment is of testable cognitive impairment is an intriguing pharmacological target and clear function in CNS homeostasis, we believe TREM2 signalling could provide neuroprotection. The expression of TREM2-DAP12 function and therapeutic potential OA Anaesthetics 2013 Jun 01;1(1):10.
committees related to the institution in which they were performed.

Microglia are the resident innate immune effectors within the CNS responsible for maintaining a healthy cellular environment. Microglia typically comprise approximately 10%–15% of total CNS cells, although estimates of the ratios of glia to neurons vary widely between studies and brain regions. In normal adult CNS, microglia sample local microenvironments for indications of injury, pathology or infection, conditions which will then cause them to classically 'activate' into an M1-like phenotype. Neuronal migration and development occurs mainly embryonically, but glial development occurs early in postnatal periods. Microglia are derived from the migration of blood monocytes, which populate and mature from ameboid monocytes into long-lived ramified microglia. After resolving pathologies, some microglia will undergo apoptosis and eventually will need to be replaced from blood-derived monocytes, but a considerable population of cells will exist continually throughout the life of an individual. Aged microglia display dysfunctional processes, while maintaining the usual hallmarks of senescent ramified cells. Loss of an intact cytoskeleton through aging results in a reduced ability to provide extracellular surveillance and will be less likely to initiate defensive mechanisms.

Volatile anaesthetics, such as isoflurane (ISO), are widely used in both young and elderly patient populations. Recent studies of the effects post exposure to commonly-used anaesthetics in young rats or mice during the sensitive period of synaptogenesis, have shown loss of synapses in the hippocampus, dendritic cytoskeletal breakdown and lasting cognitive deficits. POCD is a confounding issue for the elderly patient population, with patients 60 years and older at significant risk. ISO exposure causes upregulation of major indicators of neuroinflammation and proteins associated with Alzheimer's disease (AD). This inflammatory phenotype is hypothesized to eventually result in neuronal loss. A similar inflammatory paradigm has also been postulated for the learning impairment seen in young children who have had multiple anaesthetic exposures. ISO exposure of 1.4%–1.7% for two hours impaired the ability of aged rats to escape from a Morris Water Maze test when compared to propofol anaesthesia. Single post-ISO exposure in PND5-7 mice results in neurotoxicity through pro-BDNF-p75NTR signalling, inducing neuronal apoptosis. Sypaptic loss following ISO in these mice is attenuated when the alternative mBDNF-TrkB signalling is promoted.

Although post-ISO exposure effects on humans is a subject of considerable scientific debate, few clinical studies have provided evidence for either neurotrophic or neurotoxic effects long-term. Current theories of anaesthetic clinical mechanisms involve anaesthetics binding to and changing conformational and functional states of neuronal proteins, such as the inhibitory γ-aminobutyric acid (GABA) receptor and the excitatory N-methyl-D-aspartic acid (NMDA) receptor. These effects are transient post exposure in neurons and only partially explain the prevalence of either early learning disabilities or the estimated 10% of elderly patients developing POCD.

Although the analgesic properties of ISO are based on neuronal actions, ISO will also affect other resident CNS cell populations during exposure, potentially altering their functional states as directly as neurons. ISO exposure increases astrocyte uptake of glutamate, which may partially explain the activity changes in NMDA expressing neurons, leading to the unconscious state. More recent studies have also demonstrated significant cytoskeletal changes in immature astrocytes and cultured astrocytes after ISO exposure.

Tas et al. investigated the complex relation between cell signalling and cytoskeletal rearrangements in G6 glioma cells using stimulation of β-adrenergic receptors with isoproterenol. The stellate morphology of astrocytes, caused by the downstream activation of adenyl cyclase and increased intracellular cAMP levels, is reversed when the cells are treated with ISO, which appears to activate RhoA/Rho-kinase pathways altering cytoskeletal stability.

Although the importance of dysfunction of other non-neuronal cell types, such as astrocytes, following ISO exposure, may also be linked to childhood learning disabilities or POCD in elderly populations, some defining markers of neuroinflammation, TNF-α, IL-1β and IL-6, have been detected in both populations of patients suggestive of an inflammatory phenotype. Neuroinflammation may be defined as 'acute', occurring after injury or infection, or 'chronic', usually the result of irresolvable cyclic pathologies and is found in many neurodegenerative diseases. Microglia are implicated in the development of chronic neuroinflammatory phenotypes leading to learning impairments in neonatal rats and adult mice and the development of POCD in aged rats, via lipopolysaccharide (LPS)-TLR4 signalling and the downstream pro-inflammatory cytokine IL-1β.

Many groups have used anti-inflammatory strategies following various CNS injury models and evaluated the efficacy in preserving cognition or attenuating cognitive decline. Jenrow et al. administered MW01-2-151SRM, a selective inhibitor of pro-inflammatory cytokine production, to preserve functional dentate gyrus neurogenesis after radiation. Minocycline and memantine and a TNF-α synthesis inhibitor have all demonstrated anti-inflammatory based improvements in cognition, by...
modulating microglial activation. However, microglia activation and the definition of microglia phenotypes have become a topic of considerable research and are potentially critical to establishing preventative or palliative treatments for the neurotoxicity induced by ISO exposure.

An early attempt to use NO-Flur-biprofen, a novel NSAID in 1999, to reduce inflammation in young, adult and aged rats following LPS stimulation resulted in improved memory performance but only in the young animals, indicating temporal changes of the microglial phenotype. Once easy to categorize as resting or activated, microglia, even at homeostatic conditions, may have a continuum of phenotypic characteristics. And, more importantly, this dynamic range may change throughout the lifespan of an individual and can therefore be instrumental in either maintenance of adult neurogenesis or as the agent of neurotoxicity during inflammatory cycles.

However, in the search for potential treatments to attenuate the cognitive dysfunction in both young and aged patients post-anæsthetic exposure, microglia have been overlooked. Neuron and astrocyte cytoskeletal integrity is disrupted with ISO treatment, particularly actin stability. The actin cytoskeleton is a key regulatory point for microglia function, with critical roles in cell migration, proliferation and phagocytosis.

An emerging inflammatory and cytoskeletal mediator expressed on the membrane of monocyte-derived cells, TREM2 and the co-expressed intracellular adaptor DNAX-activating protein of 12kD (DAP12), represent an unexplored therapeutic target for treatment in either patient group. TREM2 is expressed in embryonic through aged microglia, but levels of mRNA and protein vary with development and maturation. Expression in the postnatal mouse brain reaches maximal levels in grey matter by P5 and in the corpus callosum and areas of continuing neurogenesis, such as the sub-ventricular zone by P10, suggesting these proteins are involved in synaptogenesis and synaptic pruning during early CNS development.

Head et al. have reported PND5-7 as the sensitive period in mouse hippocampal development to ISO exposure, a period of declining TREM2 expression. TREM2 positive cells are found in adult mouse brains, with both expression and regional differences. Areas of higher cognitive functions, such as entorhinal and cingulate cortex, in conjunction with medial hippocampal areas, are sites of increased TREM2 positive microglia. Studies of genetic mouse models of AD suggest that TREM2 expression declines with age and is only upregulated in areas of Aβ plaques. AD transgenic mice, Aβ, show higher rates of behavioural problems and increased inflammatory proteins after twice weekly exposure of ISO for three months (aged 7–10 months) compared to WT control mice.

A principal function of the TREM2-DAP12 signalling complex is enhanced phagocytosis by microglia without the accompanying pro-inflammatory cytokine expression seen after LPS-TLR4-NFκB stimulated signalling. Figure 1 schematically displays the initial steps in TREM2-DAP12 signalling with ligand binding, DAP12 protein–protein interactions and the non-receptor cytoplasmic tyrosine kinase Syk phosphorylation events. Loss of TREM2 changes the phenotype of microglia to a more inflammatory state and over-expression promotes phagocytosis and decreased TNF-α and IL-1β production. Actin rearrangements are required for the

Figure 1: Protein–protein interaction of TREM2 and DAP12 leads to intracellular signalling transduction. (A) TREM2 is a transmembrane protein with an extracellular binding domain, short cytoplasmic tail and positive charge. DAP12 is negatively charged with a long cytoplasmic tail and four available tyrosine residues (Y) for phosphorylation. (B) Although precise ligands for TREM2 are still unknown, once TREM2 is activated, DAP12 forms a signalling complex, which is phosphorylated by the Src kinase family (SKF) members Fyn, Lyn or Hck. Syk is also phosphorylated by SKF and once activated binds to the phosphorylated Y of DAP12.

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initial phases of phagocytosis, such as membrane ruffling and remodelling, pseudopodia extension and endocytosis of synaptic or apoptotic debris.

A recent extensive system biology/network analysis of TREM2 modules in human brain has revealed two distinct TREM2-DAP12 signalling pathways leading to actin regulation and increased microglia phagocytosis. An inhibitory pathway is also described as an alternative path through activated Ras-GTPase. Phosphorylated coflin stabilizes F-actin filaments and limits phagocytosis.

of under-performing and damaged microglia. Figure 3 is a graphical representation of the potential results of poorly functioning microglia following ISO exposure. As TREM2 expression is decreased in young and old cohorts, the microglia phenotype will be skewed towards a more inflammatory state, partially explaining the neuroinflammation seen in these groups. Reduced phagocytosis, caused by loss of TREM2, may result in failure to resolve inflammation caused by neuronal apoptosis. Reduced mobility by microglia could result in more widespread inflammation and depressed proliferation may leave the CNS more vulnerable to injury, infection and continuing unresolved inflammation.

Our hypothesis of a link between reduced TREM2-DAP12 expression and susceptibility to cognitive dysfunction following ISO exposure in these two groups is highly plausible; as adult populations with an abundance of TREM2 positive cells are not at significant risk for cognitive impairments. The concept of regulating actin polymerization to attenuate ISO cytotoxic effect is not new. Lemkul et al. demonstrated attenuation of neuronal cytotoxicity by inhibition of RhoA activation or stabilization of actin55, but therapeutic manipulation of TREM2-DAP12 signalling or expression to regulate actin is a novel idea. The benefits of driving microglia to enhanced phagocytosis without stimulating classical M1 inflammatory phenotypes would be of great interest. Currently, there are no known ligands for TREM2, so a direct agonist approach is unavailable and these sensitive patient populations have lower TREM2 expression. A more viable option may be found through mining the extensive network analysis of TREM2 biological modules to identify critical proteins involved in TREM2-DAP12 pathways to sustain microglial phagocytic functions in the sensitive populations after ISO exposure.
Cav-1 knockout thymocytes display reduced phagocytic capabilities, indicating an unappreciated role in TREM2 signalling. Figure 4A describes the putative translocation of TREM2-DAP12 and activated Syk to lipid raft-caveolae domains where higher localized concentrations of downstream partners exist to enhance transduction of signalling. Panel B of Figure 4 demonstrates how increasing caveolin expression may potentially enable increased signalling when TREM2 expression is decreased. This suggests that moderation of caveolin(s) expression may represent one such potential therapeutic target to stimulate phagocytosis in microglia through TREM2 pathways.

Conclusion
The temporal reduction of TREM2 expression mirrors the sensitive patient populations for cognitive problems after ISO exposure. Reduced TREM2 signalling results in reduced microglial induced actin-dependent phagocytosis, which may play an important factor in synaptic loss seen in the sensitive groups. Finally, increasing caveolae biogenesis or increasing the scaffolding caveolae-associated proteins, caveolins, may be mechanisms to explore potential therapeutic targets to enhance TREM2 signalling.

Abbreviations list
AD, Alzheimer’s disease; BDNF, brain derived neurotrophic factor; DAP12, DNAX-activating protein of 12kD; GABA, γ-aminobutyric acid receptor; ISO, isoflurane; LPS, lipopolysaccharide; MLR, membrane lipid rafts; NMDA, N-methyl-D-aspartic acid receptor; PM, plasma membrane; POCD, post-operative cognitive decline; TREM2, triggering receptor expressed in myeloid cells 2.

References
1. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, et al. Early exposure to anesthesia and learn-
Caveolae-lipid rafts can enhance TREM2-DAP12 signalling. (A) In unstimulated basal cells, TREM2 and DAP12 reside outside of caveolae. Caveolin, via a cytoplasmic scaffolding domain, binds and concentrates Src kinase family (SKF) members and many diverse signaling partners, such as P13K, PKC and Ras. Once stimulated by ligand, TREM2-DAP12 move into caveolae-lipid raft domains where further downstream signalling maybe initiated in a regulated manner. (B) Top panel shows schematically the consequences of reduced TREM2 availability at the PM. The spatial distribution of receptor; adaptor or lipid raft may be a physical constraint to the initiation of signalling and if a threshold of activated receptors is required for propagation of signalling, the lack of concentration into raft domains would also limit transduction. Increasing caveolin expression and/or increasing caveola biogenesis may reduce the spatial and temporal limitations of transduction.

**Figure 4:** Caveolae-lipid rafts can enhance TREM2-DAP12 signalling. (A) In unstimulated basal cells, TREM2 and DAP12 reside outside of caveolae. Caveolin, via a cytoplasmic scaffolding domain, binds and concentrates Src kinase family (SKF) members and many diverse signaling partners, such as P13K, PKC and Ras. Once stimulated by ligand, TREM2-DAP12 move into caveolae-lipid raft domains where further downstream signalling maybe initiated in a regulated manner. (B) Top panel shows schematically the consequences of reduced TREM2 availability at the PM. The spatial distribution of receptor; adaptor or lipid raft may be a physical constraint to the initiation of signalling and if a threshold of activated receptors is required for propagation of signalling, the lack of concentration into raft domains would also limit transduction. Increasing caveolin expression and/or increasing caveola biogenesis may reduce the spatial and temporal limitations of transduction.


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