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Review

Natural Compounds as a Therapeutic Intervention following Traumatic Brain Injury: The Role of Phytochemicals

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Abstract

There has been a tremendous focus on the discovery and development of neuroprotective agents that might have clinical relevance following traumatic brain injury (TBI). This type of brain injury is very complex and is divided into two major components. The first component, a primary injury, occurs at the time of impact and is the result of the mechanical insult itself. This primary injury is thought to be irreversible and resistant to most treatments. A second component or secondary brain injury, is defined as cellular damage that is not immediately obvious after trauma, but that develops after a delay of minutes, hours, or even days. This injury appears to be amenable to treatment. Because of the complexity of the secondary injury, any type of therapeutic intervention needs to be multi-faceted and have the ability to simultaneously modulate different cellular changes. Because of diverse pharmaceutical interactions, combinations of different drugs do not work well in concert and result in adverse physiological conditions. Research has begun to investigate the possibility of using natural compounds as a therapeutic intervention following TBI. These compounds normally have very low toxicity and have reduced interactions with other pharmaceuticals. In addition, many natural compounds have the potential to target numerous different components of the secondary injury. Here, we review 33 different plant-derived natural compounds, phytochemicals, which have been investigated in experimental animal models of TBI. Some of these phytochemicals appear to have potential as possible therapeutic interventions to offset key components of the secondary injury cascade. However, not all studies have used the same scientific rigor, and one should be cautious in the interpretation of studies using naturally occurring phytochemical in TBI research.

Keywords: adult brain injury; animal studies; head trauma; TBI; therapeutic approaches for the treatment of central nervous system injury

Introduction

It is now recognized that there are at least two different phases following any type of traumatic brain injury (TBI). The first is the primary injury resulting from the mechanical trauma itself. This could be the direct bruising of the tissue inside the cranial vault or the shearing of axons as the brain is forced to very quickly shift position.[1] These types of changes can only be prevented by pre-injury circumvention. What is now clear is the fact that after mechanical trauma, multiple secondary injury cascades (SIC) are initiated. If left unchecked, these cascades contribute to additional TBI-associated pathology resulting in numerous neurological problems. The clinical manifestations of TBI appear to be directly associated with the intensity of the primary injury and the magnitude/longevity of the secondary cascades. The initiation and magnitude of TBI-related SIC is a very complex process, and results in a disruption of mechanisms that normally carefully regulate multiple factors including oxidative stress, inflammation, excitotoxicity, and metabolic compromise.

The primary objective of one aspect of therapeutic management following brain trauma aims to control or decrease the development of SIC. Such efforts would lead to possible rescue of adjacent areas (penumbra) and enhance a positive outcome. This control process is complicated by the fact that other pathophysiological facets of TBI, such as enhanced cerebral perfusion pressure (CPP), intracranial pressor (ICP), and subarachnoid hemorrhage (SAH), play a role in exacerbating SIC. These factors are directly associated with the disruption of the blood–brain barrier (BBB),[2,3] providing uncontrolled exchange of ions and molecules between brain and bloodstream and access into cerebrospinal fluid (CSF).[4,5] Although disruption of the BBB is generally viewed as a negative consequence of trauma, it also allows greater access of possible therapeutic agents (especially natural compounds of large molecular size), which could aid the recovery process.

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Secondary Injury Cascades/Mechanisms

TBI is associated with a massive release of amino acids, particularly glutamic acid, which affects neurons and astrocytes, resulting in overstimulation of ionotropic and metabotropic receptors.[6,7] This condition triggers excitotoxicity events, catabolic processes, and a cellular attempt to compensate with ionic gradients and metabolic demand that increases free radical production. The excessive production of free radicals (reactive oxygen species [ROS] and reactive nitrogen species [RNS]) causes oxidative/nitrosative stress. These events not only disrupt vasculature and induce necrotic and apoptotic cell death,[8] but also contribute to brain edema.[9,10] Increased permeability of the BBB allows peripheral immune cells to activate glial cells to stimulate the production of various inflammatory related cytokines, resulting in neuroinflammation.[11] Because there are multiple different important components of the SIC, it has long been recognized that the most appropriate therapeutic approach would employ a multifaceted drug or a combinational therapy.[12] A large number of natural compounds have been reported to have multifaceted pharmacological effects that may provide neuroprotection following acute trauma.[13–18]

In medical terms, natural compounds are chemical substances produced by some type of living organism that has a medicinal and beneficial value in terms of human health. Natural compounds can be classified according to their source of occurrence, structure, or biological function. Both plants and animals may be used as a source of natural compounds/products. Plant-derived natural compounds are known as phytochemicals. Currently there are >4000 active phytochemicals that have been reported to have some type of medicinal or toxic effect. There are basically three different categories of phytochemicals: phenolic acids, flavonoids, and stilbenes/lignans. The flavonoids are the most diverse group and the most commonly used natural compounds that have been reported to provide therapeutic benefit with regard to different diseases. This review evaluates experimental animal studies that have used phytochemicals as a therapeutic intervention associated with TBI.

Breakdown of BBB and formation of cerebral edema

Although technically, breach of the BBB is part of the primary injury, it contributes to brain edema, a very early important component of SIC that is associated with any type of brain injury.[19–21] Cerebral edema is defined as an increase in brain tissue volume caused by accumulation of fluid,[22] which occurs in two basic mechanisms, vasogenic and cytotoxic.[2,3,23] Vasogenic edema results from increased BBB permeability and causes an imbalance between the oncotic and hydrostatic pressure, which regulates fluid movement between blood and brain interstitial space.[22,24] The intact BBB prevents diffusion of water-soluble molecules above 500 Da. Eventually, when the BBB is disrupted, various brain-attending proteins can be detected in the serum[25] as well as in CSF.[4,5] Cytotoxic edema is characterized by intracellular swelling of brain architect cells, while the BBB is intact. This type of edema mainly occurs in gray matter, because of energy depletion and lack of blood supply. Mechanistically, cytotoxic edema is attributed to the failure of ATPase-dependent cellular pumps and intracellular accumulation of water among osmotically active soluble ingredients.[24,26,27] Following TBI, increased glutamate can contribute to cytotoxic edema[6,7] by increasing intracellular accumulation of sodium and water osmosis, thereby increasing intracellular fluid volume.[22,28,29]

Excitotoxicity

Excitatory amino acids (EAA), a result of an increase in glutamate and aspartate, play an important role following TBI. Elevated EAA increase intracellular calcium levels ($[Ca^{2+}]i$) by mechanisms involving activation of *N*-methyl-D-aspartate (NMDA)-receptor/ion channels, the a-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor, and voltage-operated calcium channels.[30] The result is neuronal Ca^{2+}overload leading to a loss of Ca^{2+} homeostasis. Because Ca^{2+} cannot be metabolized like other second messenger molecules, cells must tightly regulate intracellular levels. There is extremely strong evidence that calcium plays an important role in the pathophysiology following TBI. As the extracellular levels of calcium decrease, the intracellular levels of calcium increase, which initiates calcium-dependent intracellular proteases. Neurons have several mechanisms of maintaining $[Ca^{2+}]i$ homeostasis including the Na^{2+}/Ca^{2+} exchanger, the endoplasmic reticulum (ER), through ER-specific Ca^{2+} -ATPase and the sequestering and release by mitochondria. Very high $[Ca^{2+}]$ can damage the structure of nucleic acids and some proteins, whereas intermediate levels can interfere with the control of specific kinases and can activate Ca^{2+}-sensitive proteases or phospholipases, causing cell damage. Failure to protect the cytosol against high levels of Ca^{2+} often leads to irreversible damage and causes 95% of cell morbidity.[22,31]

Mitochondrial dysfunction

Mitochondria are known as the powerhouse of the cell because of their unique ability to generate a large amount of adenosine triphosphate (ATP) via cellular respiration. These organelles also play a critical role in Ca^{2+} homeostasis. Mitochondrial dysfunction that occurs within minutes following TBI is an important aspect of SIC.[32,33] Normally, following any type of rapid influx of Ca^{2+}, the mitochondria act as a Ca^{2+} sink, which is critical to maintaining Ca^{2+} homeostasis. The sequestering of Ca^{2+} normally has very little effect on the synthesis of ATP. With a prolonged influx of Ca^{2+}, the mitochondria become overtaxed leading to the opening of the mitochondrial permeability transition pore (mPTP). The opening of the mPTP allows the release of Ca^{2+} as well as various low and high molecular weight components. With the opening of this pore, there are major changes in different cellular redox potentials such as depletion of $NAD(P)H_2$, glutathione (GSH), and increases in ROS that damage cell proteins and lipids. Cytochrome C, a mitochondrial intermembrane protein, which has been found to exert pro-apoptogenic activity, can be released into the cytosol following mPTP opening. Although cytochrome C itself may be insufficient to cause cell death, it acts in conjunction with other cytosolic factors (caspases) to activate apoptogenic proteases. Dysfunctional mitochondria also release pre-formed soluble apoptosis-inducing factor (AIF) that can cause nuclear apoptosis.

Oxidative stress

One of the more celebrated aspects of SIC involves the formation of oxidative stress, which can be defined as an imbalance between free radical production and their scavengers, antioxidants. Free radicals are the highly reactive atoms or molecules that have lone-paired electron(s) generated during oxidative metabolism. Often following TBI, authors will describe an increase in ROS and RNS. Examples of ROS are superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($\bullet OH$), and hydrogen peroxide (H_2O_2), whereas RNS are peroxynitrite ($ONOO^-$). Once ROS/RNS are formed, they can start a chain reaction[8] that damages key cellular components such as

lipids, proteins, and nucleic acids (DNA and RNA). Normally, various antioxidants scavenge ROS/RNS as well as inhibiting their formation.[21,34] Because of its high rate of oxidative metabolism, low antioxidant capacity, and high lipid content, the brain is extremely vulnerable to oxidative damage.[35]

Many experimental studies involving TBI stress the formation of lipid peroxidation (LP) which is a result of increased ROS levels. Common measures of LP include malondialdehyde (MDA) as measured by thiobarbituric acid reactive substances (TBARS), 4-hydroxynonenal (4-HNE), and acrolein. Free radicals also damage proteins through either a carboxylation or tyrosine nitration process. Consequently, experimental studies will report changes in protein carbonyls (PC) and 3-nitrotyrosine (3-NT) as additional measures of oxidative stress. Several studies have linked increased oxidative stress with an exacerbation of mitochondrial dysfunction[36] and cytoskeletal damage. Normally, a variety of antioxidants, both enzymatic and nonenzymatic, protect the CNS against oxidative damage, and are often evaluated as an indirect measure of changes in ROS levels. The most common are GSH, glutathione peroxidase (GPx), which converts peroxides into nontoxic forms, and glutathione reductase (GR), which reduces oxidized GSH to restore its antioxidant properties. Superoxide dismutase (SOD) is an important enzyme that reduces the superoxide burden in tissue and works in concert with catalase that can break down H_2O_2 into H_2O and O_2. Following moderate TBI with no therapeutic intervention, there is a very rapid increase in oxidative stress and a parallel decline in levels of antioxidants.[37,38] Although mitochondrial dysfunction is one of the major sources of ROS generation in the brain, other sources such as oxidation of catecholamines, extravasated substances, bradykinin formation, arachidonic acid (AA) activity, [39–41] and abundance of iron (Fe^{+++}) play a critical role.[42] Free fatty acids, AA, and LP byproducts (e.g., acrolein) can upregulate nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOX),[43] a major source of $O_2^{\bullet-}$. NOX activity and $O_2^{\bullet-}$ production are upregulated in the cerebral tissue following TBI.[44–46]

Inflammation

An integral and complex part of SIC revolves around the process of neuroinflammation, and appears to be a key element in any acute and chronic therapeutic intervention.[47] Neuroinflammation is characterized by glial cell activation, infiltration of perivascular cells (leukocytes), and subsequent expression of cytokine/chemokines.[11] This response is considered to be a double-edged ''sword'' because it can have both beneficial and detrimental consequences. Typically, the brain is protected from peripheral immune reactions because of the BBB, and has its own immune system, facilitated by glial cells.[22,48] When the BBB is compromised following TBI, blood-borne inflammatory factors gain access and mobilize inflammatory cells such as glia, which contribute to neuroinflammation. Activated microglia rapidly proliferate and migrate toward the site of injury, often sequestering the injury site.[49,50] When overactivated, microglia express a number of pro-inflammatory cytokines/chemokines such as interleukin 1β (IL-1β), tumor necrosis factor –α (TNF-α) and interferon-γ (IFNγ), which can contribute SIC.[51] Pro-inflammatory cytokines also stimulate astrocytes, causing astrogliosis.[52] Activated astrocytes can upregulate the expression of various neuroprotective trophic factors including brain-derived neurotrophic factor (BDNF), glial-fibrillary-acidic-protein (GFAP), and vimentin, and downregulate excitotoxicity, thus promoting the recovery processes. However, prolonged overactivation of astrocytes can also obstruct neuronal plasticity and present conditions that are unfavorable for axonal growth.[53]

Highly dynamic and motile microglia are spread throughout the brain parenchyma and oversee a homeostatic role. They monitor their surrounding environment for the presence of pathogenic agents or damaging processes and remove degenerative debris.[54,55] Prolonged activation of microglia is thought to be detrimental to the recovery process by exacerbating the expression of pro-inflammatory cytokines and increased neurodegenerative processes. Many experimental studies only evaluate the pro-inflammatory cytokines such as IL-1β and TNF-α. Although activated microglia express a variety of pro-inflammatory factors, such as pathogen recognition receptors,[56,57] complement receptors, cell adhesion molecules, and pro-inflammatory cytokines, they can also express anti-inflammatory factors that can downregulate the immune response and calm microglia in a normal (nonpathologic) condition.[58] In addition to glial cells, migrated leukocytes such as T cells, monocytes, and neutrophils from the blood also contribute to inflammation in the brain.[59–62]

Natural Compounds and Their Treatment Effects after TBI

For this review, we have only included published peer-reviewed papers that have investigated a plant-derived natural compound as a therapeutic intervention following experimental TBI. Work involving synthetic experimental compounds and work dealing with vitamins have not been included. Experimental TBI is defined as brain injury resulting from a mechanical device developed to explore secondary injury components of blunt force trauma. Experiments involving ischemia or non-impact-related ablations have not been included. Experiments dealing with blast injury models have not been included. In addition, studies of brain injury as a consequence of a neurological disorder in an animal model (e.g., Huntington's disease) were not included. This review is further limited to work with either young adult rats or mice, and studies involving neonates or aged animals have not been included. Although there are many different approaches for this type of review, we have decided to alphabetically list the different phytochemicals that have been investigated and to summarize the major findings in those studies. Table 1 summarizes the various effects different phytochemicals have following experimental TBI.

Allicin

Allicin (diallyl thiosulfinate) is a compound found in garlic that provides its typical aroma when crushed. Garlic has been recognized as a folk medicine for centuries and has a variety of therapeutic effects including anti-inflammatory and anti-hypertensive activities. A recent study evaluated allicin as a therapeutic intervention following a mild CCI injury in rats.[63] This set of experiments explored both a dose-response and an effective therapeutic window for the natural compound. The most effective dose was 50 mg/kg, which altered a variety of secondary injury cascades including oxidative stress, neuroinflammation, apoptosis, and improved the TBI-related declines in neuroscores. The therapeutic window was reported to be between 2 and 4 h post-trauma. It was hypothesized that the neuroprotective effects of allicin were primarily related to activation of the Akt/endothelial nitric oxide synthase pathway. Nitric oxide (NO) is catalyzed from the nitric oxide synthase enzymes (NOS) endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). All three of the isoforms are important components of TBI-related SIC. Although the exact roles of these three isoforms is unclear in terms of neuronal survival, it is believed

Table 1. Summary of Key Dependent Variables in Animal Studies Evaluating Phytochemicals as a Therapeutic Intervention after TBI

Compound/Study	*Species*	*Timing of treatment*	*No. Treatments*	*Edema*	*Oxidative stress*	*Inflammation*	*Cell loss*	*Lesion volume*	*Modified neuroscore*	*Morris Water Maze*
Allicin										
Chen et al.[63]	? rats	Post	Single	+	+	+	TUNEL +	+	+	
Apocyn										
Choi et al.[45]	SD rats	Pre	Single		+	+	FJB +			
Zhang et al.[46]	CD-1 mice	Pre/post	Single		+	+	Cresyl +			
Song et al.[67]	SD rats	Pre	Single	+	+					+
Ferreira et al.[69]	Swiss mice	Pre/post	Multiple	−	+	+		+	+	
Loane et al.[68]	C57 mice	Post	Single		+			+	+	+
Lu et al.[66]	ICR mice	Pre	Single		+		TUNEL +		+	
Baicalein										
Chen et al.[71]	SD rats	Post	Single/multiple			+	FJB +	+	+	
Wang et al.[72]	SD rats	Post	Multiple	+		+	TUNEL +		+	
Caffeic acid										
Kerman et al.[75]	SD rats	Post	Single		+					
Zhao et al.[76]	SD rats	Post	Multiple				Cresyl −	+	+	−
Caffeine										
Al Moutaery el al.[78]	Wistar rats	Pre	Single	−	−		TUNEL −		−	
Dash et al.[81]	SD rats	Post	Single					+	−	−
Li et al.[80]	Kunming mice	Pre	Single	+		+	TUNEL +		−	
Lusardi et al.[79]	SD rats	Post	Single					−	+	
Cocaine										
Muir and Ellis[82]	SD rats	Pre	Single							
Muir et al.[83]	SD rats	Pre	Single						−	−
Colchicine										
Gahm et al.[89]	SD rats	Post		−			FJB + TUNEL −			
Coumarin										
He et al.[91]	SD rats	Pre	Single	+	+		Cresyl − TUNEL +	+	+	
Crocin										
Wang et al.[92]	C57Bl6 mice	Pre	Single	+		+	TUNEL +		+	
Curcumin										
Wu et al.[97]	SD rats	Pre	Diet		+					+
Sharma et al.[96]	SD rats	Pre	Diet							
Sharma et al.[95]	SD rats	Post	Diet		+					+
Wu et al.[98]	SD rats	Post	Diet		+				+	+
Laird et al.[100]	CD-1 mice	Pre/post	Single	+		+		−	+	
Samini et al.[99]	Wistar rats	Pre	Multiple		+			+	+	
Zu et al.[101]	C57Bl6 mice	Post	Single	+		+	FJB + TUNEL +		+	

(*continued*)

TABLE 1. (CONTINUED)

Compound/Study	*Species*	*Timing of treatment*	*No. Treatments*	*Edema*	*Oxidative stress*	*Inflammation*	*Cell loss*	*Lesion volume*	*Modified neuroscore*	*Morris Water Maze*
7,8-dihydroxyflavone										
Wu et al.[103]	C57Bl6 mice	Post	Multiple	+			FJB + TUNEL +	+	−	
Chen et al.[104]	C57Bl6 mice	Pre	Single				FJB +			
Zhao et al.[105]	C57Bl6 mice	Post	Multiple					−	+	+
Agrawal et al.[108]	SD rats	Post	Multiple						+	
Zhao et al.[106]	C57Bl6 mice	Post	Multiple							
Alder et al.[107]	C57Bl6 mice	Post	Single				FJB +		−	−
Ellagic acid										
Farbood et al.[110]	Wistar rats	Pre	Multiple			+			+	
Epigallocatechin gallate										
Itoh et al.[111]	Wistar rats	Pre	Diet		+		NeuN +			+
Itoh et al.[112]	Wistar rats	Pre	Diet		+					
Itoh et al.[113]	Wistar rats	Pre/post	Diet		+					Pre + Post −
Formononetin										
Li et al.[114]	Wistar rats	Post	Multiple	+	+			+	+	
Gallic acid										
Sarkaki et al.[115]	Wistar rats	Pre	Multiple			+			+	
Genistein										
Hong et al.[116]	SD rats	Post	?							
Soltani et al.[117]	Wistar rats	Post	Multiple	+					+	
Ginkgo biloba										
Hoffman and Stein[120]	SD rats	Pre/post	Multiple				Thionin +		+	+
Menku et al.[121]	Swiss rats	Post	Multiple	−	+					
Yu et al.[122]	Wistar rats	Post	Multiple			+	TUNEL +			
Ginseng										
Ji et al.[123]	SD rats	Post	Single				TUNEL −		+	
Xia et al.[124]	SD rats	Post	Multiple	+	+	+	TUNEL +	−	+	
Kumar et al.[126]	Wistar rats	Post	Multiple		+	+				+
Chen et al.[125]	Wistar rats	Post	Single	+					+	
Hydroxysafflor yellow A										
Bie et al.[127]	SD rats	Pre	Multiple		+		TUNEL +			
Lovastatin										
Chen et al.[128]	SD rats	Pre	Multiple			+	FJB +	+	+	
Luteolin										
Xu et al.[129]	ICR mice	Post	Single	+	+		TUNEL +		+	
Xu et al.[130]	ICR mice	Post	Single	+		+	FJB +		+	
Sawmiller et al.[131]	Tg2576 mice	Pre	Multiple							
Cordaro et al.[132]	CD-1 mice	Post	Single	+	+	+	H&E +		+	

(*continued*)

Table 1. (Continued)

Compound/Study	Species	Timing of treatment	No. Treatments	Edema	Oxidative stress	Inflammation	Cell loss	Lesion volume	Modified neuroscore	Morris Water Maze
Morphine										
Robinson et al.[138]	SD rats	Pre	Single							
Hayes et al.[140]	SD rats	Pre	Single						+	
Lyeth et al.[139]	SD rats	Pre	Single						+	
Statler et al.[141]	SD rats	Post	Single				FJB −	+	−	−
Zohar et al.[142]	ICR mice	Post	Single							+
Naringin										
Cui et al.[144]	Wistar rats	Pre/post	Multiple	+	+	+			+	
Nicotine										
Verbois et al.[145]	SD rats	Post	Multiple					+		
Verbois et al.[146]	SD rats	Pre/post	Multiple					−		Pre − Post +
Shin et al.[147]	SD rats	Post	Multiple							
Nitidine										
Yuan et al.[148]	C57Bl6 mice	Post	Single			+	TUNEL +			
Puerarin										
Wang et al.[149]	SD rats	Pre	Single		+	+	FJB +			
Pycnogenol										
Scheff et al.[150]	SD rats	Post	Multiple		+	+				
Ansari et al.[151]	SD rats	Post	Multiple		+					
Norris et al.[152]	SD rats	Post	Single							
Scheff and Roberts[153]	SD rats	Post	Multiple				FJB −	+		−
Quercetin										
Schultke et al.[154]	SD rats	Post	Multiple		+					
Yang et al.[155]	SD rats	Post	Multiple		+	+	TUNEL +			+
Resveratrol										
Ates et al.[159]	Wistar rats	Post	Single	+	+			+		
Singleton et al.[161]	SD rats	Post	Multiple				Cresyl +	+	+	+
Gatson et al.[160]	C57Bl6 mice	Post	Multiple		+					
Lin et al.[162]	SD rats	Post	Single		+					
Feng et al.[163]	SD rats	Post	Multiple	+		+			+	+
Feng et al.[164]	SD rats	Post	Multiple	+					+	+
Rutin										
Kumar et al.[167]	Wistar rats	Post	Diet		+	+				+
Salvianolic acid B										
Chen et al.[168]	C57Bl6 mice	Post	Single	+		+		+	+	+
Triptolide										
Lee et al.[170]	SD rats	Post	Single	+		+	TUNEL +	+	+	
Wogonin										
Chen et al.[171]	C57Bl6 mice	Post	Single	+		+	FJB + TUNEL +	+	+	

+, positive significant effect; −, no significant positive effect or change; Cresyl (cresyl violet staining); Diet (compound delivered in the diet); FJB (Fluoro-jade staining); H&E (hematoxylin and eosin staining); Multiple (compound given multiple times or continuously); Pre (compound administered prior to injury); Post (compound administered after injury); SD (Sprague Dawley); Single (compound administered only once); Thionin (thionin staining); TUNEL, terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (staining for apoptosis).

that activation of eNOS phosphorylation is beneficial, whereas iNOS activation is detrimental.[64, 65]

Apocynin

Apocynin (4-hydroxy-3-methoxy-acetophenone), also known as acetovanillone, is a natural compound isolated from the root of Canadian hemp (*Apocynum cannabinum*) and the Chinese medicinal plant Kutki (*Picrorhiza kurroa*), which has been used for centuries in the treatment of inflammatory diseases. Several studies in both adult mice and rats have evaluated apocynin as an intervention following moderate levels of TBI. Early studies pretreated either mice[46,66] or rats[45,67] with different amounts of apocynin (Sigma-Aldrich) that range from 4 to 100 mg/kg using the i.p. route. Post-trauma interventions have used 5 mg/kg i.p.[68] or a range of 0.05– 5 mg/kg s.c.[69] in mice. Because of apocynin's known ability to inhibit NOX activity, most of the studies monitored this dependent variable following TBI. Apocynin can, however, decrease other types of oxidative stress such as protein carbonyls and lipid peroxidation. In addition, this natural compound has shown significant neuroprotection as evidenced by a reduction in terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) staining, Fluoro-jade B (FJB)-positive neurons, and lesion volume. Multiple studies have described positive effects in post-injury cognitive ability including the Morris Water Maze (MWM) and a standardized neuroscore. One of the exceptions is the study by Ferreira and colleagues[69] that failed to demonstrate any significant effect of apocynin on brain edema or injury-induced motor dysfunction. The effective therapeutic window for the use of apocynin post-trauma has not been fully explored. This literature is somewhat difficult to interpret, because many different injury models have been used without regard to a consistency in dependent variables or specific dose of the compound. The primary mechanism is cited as inhibition of NOX. It is well known that NOX is upregulated following TBI, and coincides with the excessive production of ROS.[70]

Baicalein

Bioflavonoid baicalein (5,6,7-trihydroxy-2-phenyl chromen-4-one) is extracted from the roots of the herb Baikal skullcap (*Scutellaria baicalensis*) and American skullcap (*Scutellaria lateriflora*). This flavone is a traditional medicine used in treating inflammatory and allergic diseases. Baicalein has been investigated as a therapeutic intervention in adult rats following experimental trauma, and has been shown to exert various biological activities including antioxidant, anti-inflammatory, and neuroprotective effects. In an early study,[71] rats were subjected to a severe controlled cortical impact (CCI) injury and given purified baicalein (30 mg/kg) i.p. immediately following trauma, with some animals receiving additional daily injections for 4 days. This compound demonstrated significant improvement in a variety of motor and neuroscore tasks coupled with a reduction in lesion volume and FJB staining. Baicalein also showed a significant reduction in markers of neuroinflammation. There was no significant difference between the single and multiple dose paradigms. The authors did report that the single injection was neuroprotective for as long as 28 days. A recent study evaluated the use of baicalein following a subarachnoid hemorrhage model,[72] a condition that often occurs following TBI and may be relevant to the development of novel therapeutic interventions. Treatment initiated at 30 min post-injury using either 30 mg/kg or 100 mg/kg of baicalein (Sigma) reduced BBB permeability, edema, and neuronal apoptosis, and improved the subject's neuroscore. This group also reported a significant decrease in the toll-like receptor 4 (TLR4) and nuclear factor-κB (NF-κB) as a possible mechanism. Various markers of neuroinflammation were also shown to be reduced using a 30 mg/kg dose. TRL4 and NF-κB, an inducible transcription factor, are found in both neurons and glia, and play important roles in the brain, especially in inflammation.[73,74] A possible therapeutic window for this compound was not evaluated.

Caffeic acid phenethyl ester (CAPE)

CAPE is a naturally occurring phenolic compound that is a derivative of caffeic acid (3,4-dihydroxycinnamic acid). It is found in various vegetables and fruits and can be isolated from honeybee propolis. It has been used for many years as a folk medicine because of its potent antioxidant, anti-inflammatory, and immunomodulatory character. Recently, CAPE has been tested for protective effects in TBI. Adult male rats were treated with CAPE (10μmol/kg) i.p. immediately following a diffuse injury using a closed head injury weight-drop paradigm.[75] Animals treated with CAPE demonstrated a significant reduction in lipid peroxidation and enhancement of key antioxidants compared with a vehicle-treated cohort. CAPE-treated subjects also showed a reduction in TUNEL staining in the frontal cortex. Possible changes in cognition were not explored. In another study,[76] adult male rats were subjected to a mild to moderate CCI-induced TBI, and given CAPE therapy (10 mg/kg, i.p.) initiated 30 min post-injury, with some animals treated for an additional 4 days. Rats evaluated 24 h after a single treatment demonstrated reduced BBB permeability and reduced cortical cell loss. However, CAPE treatment even for an additional 4 days failed to demonstrate a beneficial effect in spatial memory or a reduction in hippocampal cell loss. The hippocampal cell loss is surprising, given the apparent neuroprotection of cortical neurons that are directly impacted. There was no attempt to evaluate a therapeutic window for this natural compound. CAPE has been reported to exert neuroprotection through the modulation of heme oxygenase-1 and also BDNF.[77]

Caffeine

Caffeine (1,3,7-trimethylxanthine) is a well-known psychostimulant compound with its main source, the coffee plant (*Coffea arabica*). It is one of the most common addictive natural compounds consumed by a large population worldwide. From a pharmacological perspective, caffeine is very complex and known to modulate adenosine receptors (subtypes A_1 and A_{2A}). At extremely high levels, caffeine is toxic. One of the earliest TBI studies using a closed head weight-drop model, adult rats were pretreated with various doses of caffeine (50, 100, 150 mg/kg).[78] Animals treated with high doses of caffeine showed increased mortality. Other dependent measures, such as neuroscore, edema, and histopathology, were all significantly worse with the caffeine treatment. A subsequent study[79] using a single post-trauma treatment of caffeine (25 mg/kg, i.p.) following a severe lateral fluid percussion (LFP) injury reported a significant reduction in mortality and marginal improvement in the neuroscore without any significant change in histopathology. Prior chronic caffeine was investigated in adult mice[80] and compared with a single pretreatment at various doses (5, 15, 50 mg/kg, i.p.). This study failed to show any positive effects with the acute pretreatment, but did report significant improvement in neuroscore, edema, apoptosis, and inflammation with chronic 3 week pretreatment (0.25 g/L) in the subject's drinking water. In a rather unusual study, caffeine was paired with ethanol (caffienol) and given i.p. 15 min post-trauma.[81] These investigators failed to find any improvement in either motor skills or cognitive ability

following the therapy. The only positive outcome was a reduction in lesion volume at 28 days post-injury.

Cocaine

Cocaine is an alkaloid found in coca (*Erythroxylum coca*) leaves that have been part of the diet of various indigenous communities for centuries. Cocaine is considered as a hallucinogenic, addictive, and toxic compound that may also have local anesthetic ability. Relatively few experimental studies have evaluated the effects of this natural compound following TBI. Two different rat studies by the same group evaluated whether or not pretreatment with cocaine could alter the outcome following a LFP injury.[82,83] In both studies, cocaine when injected i.v. failed to produce any positive effects, including any improvement in cognitive function. Rats administered cocaine did worse on the MWM, than saline controls. A more recent study evaluated whether or not cocaine intoxication prior to TBI had an influence on several physiological measures associated with the trauma.[84] Immature miniature swine were administered cocaine 4 mg/kg and subjected to a LFP injury. The cocaine intoxication had little to no effect on any of the physiological parameters. There have also been several reports involving human subjects.[85–88] All of these studies primarily focused on whether or not cocaine addiction worsened TBI outcomes.

Colchicine

Colchicine is isolated from colchicum plant (*Colchicum autumnale*). At higher doses it is a toxic compound. Because of its anti-inflammatory potential, colchicine has been used to treat various inflammation mediated diseases; for example, rheumatic arthritis (gout). There is a single study in the literature evaluating the effects of post-trauma treatment with this natural compound.[89] Four hours following a moderate to severe cortical injury using the Feeney's weight-drop model,[90] adult rats were treated with colchicine (0.2 mg/kg; i.p.). The animals were given daily injections for 14 days and subsequently evaluated for changes in histopathology and iNOS. The colchicine group was compared with other cohorts treated with dexamethasone, tirilazad mesylate, or nimodipine. Although colchicine decreased the number of FJB-positive cells at 24 h post-trauma, it had little effect on TUNEL staining. Mechanistically, its neuroprotection appears to be linked to a decrease in levels of iNOS.

Coumarin

Coumarin is found in many plants such as vanilla grass, sweet grass, and cassia cinnamon (*Cinnamomum cassia*), which is very different from true cinnamon. Osthole is a compound that can be isolated from the coumarin containing plant, *Cnidium monnieri*, which has been used in traditional Chinese medicine. A recent study demonstrated that osthole (20 mg or 40 mg/kg, i.p.), when administered 30 min prior to a moderate TBI using the Feeney model,[90] could provide neuroprotective effects in the adult rat.[91] Animals pretreated 30 min prior to injury demonstrated decreased neurological deficits consisting of prehensile traction and beam-balancing at 24 h post- injury. This natural compound was also shown to significantly reduce cerebral edema and hippocampal CA3 neuronal loss, with the greatest effects observed at 40 mg/kg at 24 h post- trauma. This same pretreatment significantly reduced oxidative stress and enhanced antioxidant levels (GSH, SOD) in the injured cortex. Levels of apoptosis in the cortex, as evidenced by TUNEL staining, were also significantly reduced with the highest dose of the derivative, osthole. It is unfortunate that this unique natural compound was not evaluated as a post-injury therapeutic. There is no clear mechanism cited by the authors other than the fact that it has antiapoptotic and antioxidative properties.

Crocin

Crocin is a pharmacologically active component of saffron (*Crocus sativus L.*), which has been used for centuries as a herbal remedy for various diseases, including neurological problems. In a recent study using a moderate rodent CCI model of TBI in mice, crocin (20 mg/kg, i.p.) was administered 30 min prior to the injury.[92] Crocin administration significantly decreased the neurological motor severity score[93] and brain edema at 24 h post injury. These authors also reported a modest reduction in markers of neuroinflammation coupled with a significant decrease in cortical TUNEL-positive cells. The proposed mechanism of action was activation of the notch signaling pathway.[94]

Curcumin

The bioflavonoid, curcumin, is a polyphenolic compound that occurs in the spice turmeric (*Curcuma longa L.*). There are a number of research studies suggesting that curcumin has significant healing effects, possibly because of its strong anti-inflammatory and antioxidant potential. In a series of studies from the same laboratory,[95–98] adult rats were subjected to a mild LFP experimental injury and either pretreated for 4 weeks with a diet containing curcumin (500 ppm) or post-treated for 2 weeks. The 4 week pretreatment reduced oxidative stress, increased BDNF levels, protected synaptic proteins, protected mitochondria, and showed a moderate effect on MWM performance. Post-treatment for 2 weeks had a much more dramatic effect on oxidative stress, mitochondrial homeostasis, and MWM performance. A subsequent adult rat study[99] tested pretreatment of curcumin using either a 50 or 100 mg/kg i.p. injection. Rats were subjected to a cortical contusion using the Feeney weight-drop method[90] and tested for SIC type changes. Curcumin at both doses significantly improved locomotor behavior. Cortical lesion volume was marginally improved only at 100 mg/kg. Lipid peroxidation was also only marginally improved with the100 mg/kg dose. No attempt was made to assess post-injury treatment. In a mouse study using a moderate to severe injury, both pretreatment (75 mg/kg, 150 mg/kg i.p.) immediately before the injury or 300 mg/kg i.p. at 30 min post-injury, was significantly effective in reversing some of the SIC.[100] Pretreatment with curcumin or treatment within the first 30 min post-injury improved both open field and novel object behavior. It was also effective in reducing neuroinflammation and edema and blocked IL-1β aquarpoin-4 expression. However, there was virtually no neuroprotection, because cortical lesion size was unaffected. A recent study also evaluated curcumin following a severe cortical injury using a modified Feeney model.[101] Adult mice were injured and treated 15 min post-trauma with 100 mg/kg curcumin i.p. This natural compound showed marginal but significant reductions in neuroinflammation, edema, TUNEL staining, and FJB staining. It also improved the neuroscore at 24 h post-injury. The proposed mechanism of action is very similar to that of baicalein, involving inhibition of TLR4 and NF-κB.[73,74]

7,8-Dihydroxyflavone (7,8-DHF)

7,8-DHF is a bioflavonoid found in *Godmania aesculifolia*, *Tridax procumbens*, and primula tree leaves that can protect against oxidative stress and excitotoxicity-induced neuronal degeneration. It is a

small molecule that easily crosses the BBB and binds to tyrosine receptor kinase B (TrKB) as an agonist, and mimics BDNF.[102] Recently, several studies evaluated the therapeutic efficacy of 7,8-DHF in TBI. The majority of the studies have been conducted using young adult mice. One of the early investigations evaluated mice following a severe CCI injury.[103] Initial experiments determined that 20 mg/kg i.p for 3 days post-injury was more effective than 50 mg/kg. 7,8-DHF improved rotarod performance, beam walking, and the overall neuroscore but not during the 1st week. It also had a significant effect on edema. The neuroprotective properties, as evaluated by FJB and TUNEL staining, were significant but not robust. There was a reduction in lesion volume at 28 days. This natural compound increased BDNF levels post-injury. 7,8-DHF was also evaluated as a pretreatment using a much lower dose (5 mg/kg).[104] Young adult mice were pretreated 1 h prior to a moderate to severe injury and evaluated 24 h post-trauma. There was a significant reduction in FJB staining in the hippocampal granule cell layer. It is somewhat surprising that very few positive cells were observed in the CA3 region, even in the vehicle treated subjects. There was also a greater number of doublecortin-positive cells, indicative of increased survival of immature neurons. This same group has also tested 7,8-DHF (5 mg/kg; i.p.) as a post-injury treatment.[105,106] In an additional study, mice were subjected to the same moderate CCI, and the 7,8-DHF therapy initiated 1 h post- trauma with daily injections continued for 2 weeks. This treatment again increased the survival of post-trauma neurogenesis in the hippocampus, with the added feature of enhancing dendritic arborization. Another study explored whether or not post-treatment lasting only 3 days was sufficient to improve both morphology and behavior after the trauma. This therapeutic regime with 7,8-DHF resulted in enhanced dendritic morphology in the residual injured cortex, although it did not significantly reduce neuronal death. There was also a moderate enhancement in MWM performance and a moderate early improvement in rotarod performance. Employing a LFP injury and a single dose of 5 mg/kg at 2 h post-trauma, adult mice were tested in the MWM beginning at 1 day post-injury.[107] The compound failed to show any significant improvement in MWM latency. It also failed to show any improvement in rotarod behavior even at 21 days post- trauma. There was a significant suppression of caspases in the cortex but not in the hippocampus. FJB staining was reduced in both the cortex and the hippocampus. Somewhat surprising is the fact that sham animals treated with 7,8-DHF showed considerable FJB staining in the hippocampus. The single adult rat study used a moderate/severe LFP injury coupled with a 5 mg/kg i.p. dose of 7,8-DHF.[108] Rats were treated once daily for 7 days and evaluated for cognitive performance and markers of brain plasticity. Subjects treated with 7,8-DHF were comparable with sham on the Barnes maze test. There were also increased levels of several markers of brain plasticity such as growth-associated protein-43 (GAP-43) and cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) phosphorylation. None of the studies have run a dose-response curve or actually evaluated a possible time course study. Activation of the BDNF receptor tropomyosin-related kinase B (TrkB) is a possible mechanism for the abovementioned beneficial effects of 7,8-DHF, which then plays an important role in mitochondrial homeostasis.

Ellagic acid (EA)

EA (2,3,7,8-tetrahydroxybenzopyranol[5,4,3-cde]benzopyran-5-10-dione) is a natural polyphenolic antioxidant found in various fruits and nuts. Several studies have shown that EA has a variety of pharmacological effects, including anti-inflammatory, antioxidant, and immunomodulatory properties. A recent study evaluated the possible therapeutic effects of EA following a mild/moderate diffuse injury (Marmarou model[109]) in young adult rats. Animals were pretreated for 7 days by gavage with EA (100mg/kg). This pretreatment strategy reduced BBB permeability and also decreased pro-inflammatory cytokines (IL-1β and IL-6). Electrophysiology demonstrated improved long-term potentiation (LTP) in the hippocampus. This was further supported by excellent performance on a passive avoidance task.[110] The possible mechanism behind these favorable outcomes is unclear.

Epigallocatechin-3-gallate (EGCG)

EGCG is an ester of epigallocatechin and gallic acid, a type of catechin that occurs in various fruits and vegetables, and one of the major polyphenolic compounds found in green tea. This natural compound has been used in a variety of Chinese medicines to regulate hormone levels. One laboratory has conducted three separate experimental studies evaluating the possible neuroprotective effects of EGCG following a very mild TBI. In the initial study,[111] adult rats were administered EGCG in the drinking water (0.1% w/v) for 4 weeks, and subsequently given a very mild injury. This treatment significantly reduced lipid peroxidation, DNA damage, and apoptosis factor Bcl2 protein. It also improved neuronal survival and cognitive performance in the MWM. What is unusual about these results is the magnitude of the effect of the natural compound and the lack of variance within each group. The cognitive deficit observed in the MWM for such a mild injury is unprecedented in the literature. The second study[112] used an identical paradigm with the exception that this group monitored possible changes in neural stem cells in close proximity to the injury site and showed a significant increase following EGCG therapy. Many of the variables monitored in the previous study were also evaluated, and the results are identical as the previous study including the lack of variance. The third and final study[113] evaluated an abbreviated time course for EGCG treatment. Animals treated either continuously or only as a pretreatment for 4 weeks demonstrated significantly greater cognitive improvement and histological improvement than those receiving a limited post-trauma treatment. Based upon the magnitude of the positive effects, it is surprising that this compound has not been further evaluated. The mechanism of action appears to be its free radical scavenger properties.

Formononetin (FN)

FN is a phytoestrogen isoflavone found in a variety of plants and herbs such as red clover (*Trifolium pratense*). Among its many pharmacological properties are its ability to offset oxidative stress and the promotion of angiogenesis. A recent study investigated the possible beneficial properties of FN following TBI.[114] After a closed head injury, FN treatment (10 or 20 mg/kg; i.p.) 5 days post-TBI significantly increased levels of antioxidant enzymes (GPx and SOD) and reduced LP. This natural compound also improved the neuroscore and reduced brain edema, although it is unclear when this was evaluated. There was no attempt to explore a dose-response curve or probe the effectiveness at <5 days of treatment. The mechanism of action is unclear.

Gallic acid (GA)

3,4,5-Trihydroxybenzoic acid, GA, is a type of organic acid (phenolic acid) found in various fruits and vegetables, including flax seed and gall nuts. The synthetic n-alkyl esters of GA, known

as gallates, are widely used as antioxidants by the food and pharmaceutical industries. GA has a wide range of biological functions including antioxidant, anti-inflammatory, and anti-tyrosine activity. The protective effects of GA following head trauma have been recently reported using a modified Marmarou's weight-drop rat model[109] of TBI.[115] Adult rats were treated with GA (100 mg/kg) for 7 days before and 2 days after injury using oral gavage. GA significantly improved the neuroscore and passive avoidance behavior. Although the natural compound therapy improved multiple aspects of hippocampal physiology, it was less than sham operates including LTP. The levels of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 were significantly reduced in GA-treated rats but not equivalent to sham operates. The mechanism of action following TBI is unknown.

Genistein

Genistein (5,7-Dihydroxy-3-[4-hydroxyphenyl] chromen-4one) is an isoflavone compound that occurs in various plants, such as soy (*Glycine max*), alfalfa (*Medicago sativa*), lupine (*Lupinus*), chickpea, and some legumes (*Leguminosae*). Genistein is also considered to be a phytoestrogen and has been used in some hormone replacement therapies. An early study evaluated genistein's effects after a moderate fluid percussion (FP) injury in adult rats. This natural compound maintained cerebral blood flow, vasodilation, and ICP.[116] One recent study investigated the possible protective effects of genistein following a closed head injury in rats.[117] A severe brain trauma was induced with the Marmarou model.[109] Genistein therapy (15 mg/kg; i.p.) was initiated at 30 min post-trauma with a supplemental dose at 24 h. This therapy significantly reduced both brain edema and opening of the BBB at 48 h post-trauma along with a reduction in ICP. Animals treated with genistein demonstrated a complete return of motor function, unlike vehicle-treated controls. It is unfortunate that no histopathology was provided to demonstrate possible neuroprotective qualities in either study. The mechanism of action may be related to an inhibition of tyrosine kinase activity, which has an effect on cerebral blood flow.

Ginkgo biloba extract (GBE)

GBE is derived from the leaves of the ginkgo or maidenhair tree, which belongs to the plant family Ginkgoaceae. The extract of ginkgo biloba, also called as EGb 761, is believed to have a variety of medicinal purposes including CNS-related effects.[118,119] An early study evaluated EGb 761 in young adult rats as a post-injury therapy following a moderate injury to the frontal cortex using a pneumatic impactor.[120] Animals were given an i.p. injection (100 mg/kg) immediately post-injury, and for an additional 7 days. Rats injured and treated with the extract showed improvement in the MWM compared with a vehicle-treated cohort, although they still performed worse than uninjured controls. However, they showed increased cell numbers in the medial dorsal thalamus. This was coupled with a significant reduction in astrocytes and microglia. Another group also evaluated a possible therapeutic intervention with EGb 761(50mg/kg) i.p. in young adult rats following a moderate TBI using the Marmarou model.[121] Animals were treated at 1 h, with supplemental injections at 9 and 17 h. MDA levels were significantly reduced with the GBE, although brain edema levels continued to be significantly increased compared to sham operates. There was no mention of any evaluation of cognitive function. More recently, adult rats were treated with a major component of EGb 761, ginkgolide B, following a moderate to severe injury using the Feeney weight drop model.[122] Animals were treated immediately following the injury with one of three different doses (5, 10, 20 mg/kg) and then once daily until killed. The derivative significantly reduced TLR-4 and NF-κB expression as well as markers of neuroinflammation and apoptosis (TUNEL). The 5 mg/kg dose did not have a significant effect, and the 10 mg/kg and 20 mg/kg doses were equivalent. The possible mechanism of action is simply stated as inhibition of neuroinflammation and oxidative stress.

Ginseng

Ginseng, the root of *Panax ginseng* C.A. Meyer, has been used as a traditional medicine for thousands of years in Asia, and is currently gaining popularity worldwide. Many studies have evaluated the triterpenoid saponins (ginsenoside) components of ginseng, the main bioactive ingredient. There are more than 30 different ginsenoides that have been identified chemically, each with its own unique structure. Some of these ginsenoides have been casually linked to various beneficial activities, including improvement in cognitive function. Despite its historic reputation, relatively few studies have investigated the possible therapeutic benefit of ginseng with regard to TBI. One of the earliest studies evaluated ginseng total saponins (GTS) immediately following a moderate TBI using the CCI model.[123] Young adult rats were given GTS (100 or 200 mg/kg) i.p. immediately post-trauma and evaluated 24 h later. The GTS improved the neuroscore but only marginally. Lesion volume was significantly reduced, and the GTS protected neurons in the ipsilateral hippocampus. These authors failed to observe any change in cortical TUNEL staining. A more detailed study also evaluated the beneficial effects of GTS with the Feeney model in the adult rat.[124] In these studies, animals were treated twice a day for 14 days. A dose-response curve demonstrated that 20 mg/kg i.p. was the most beneficial following a moderate injury. This therapeutic regime resulted in a significant improvement in neuroscores. Although there was no change in brain edema at 24 h or in lesion volume, GTS treatment did protect neurons in the hippocampal CA3 region and significantly reduced TUNEL staining in the cortex. Markers of oxidative stress and neuroinflammation were also significantly reduced following GTS therapy. A time course evaluation showed that GTS administered up to 6 h post-trauma was effective. A relatively recent study evaluated the possible protective effects of one of the active components in ginseng in Wistar rats following a moderate TBI. A dose-response study investigated ginsinoside Rb1(GS-Rb1) following a mild CCI injury in adult rats.[125] They reported that GS-Rb1 at 20–40mg/kg i.p., given immediately following the injury, resulted in a significant reduction in brain infarction and edema, with a significant improvement in neuroscores. The most significant findings investigating the therapeutic properties of ginseng reported that even at 2 weeks after significant trauma, oral ingestion of total ginseng (100 or 200 mg/kg) eliminated oxidative stress and neuroinflammation in adult rats subjected to a severe injury using a modified Marmarou injury model.[126] Subjects were given therapeutic intervention beginning at day 14 post-trauma, with a daily oral dose of ginseng suspended in carboxymethyl cellulose solution. After treatment for 9 days, the injured rats were tested in the MWM. Rats treated with either 100 or 200 mg/kg of ginseng performed significantly better than vehicle- treated rats on day 4 of testing. Even greater results were observed when the ginseng was paired with minocycline, a non-natural microglial inhibitor. These same animals also had significant reductions in oxidative stress, increases in antioxidants (GSH, SOD, catalase), and significant decreases in several measures of neuroinflammation. It is somewhat surprising

that there are no clinical trials underway with this type of supporting data. There is no specific mechanism reported for the positive findings, other than that ginseng inhibits oxidative stress.

Hydroxysafflor yellow A (HSYA)

Hydroxysafflor is a main component in the flower of the safflower plant (*Carthamus tinctorious* L.), which is used in traditional Chinese medicine for the treatment of cardiovascular and cerebrovascular diseases. In an experiment using adult male rats, the neuroprotective properties of HSYA were investigated following a moderate level of trauma using a modified weight-drop closed head injury model.[127] Animals were pretreated 30 min prior to and 6 h after injury with 1, 2, or 4 mg/kg of HYSA i.v. The most significant reduction in contusion volume was obtained with 4 mg/kg, and this dose was subsequently further evaluated. Mitochondrial function was significantly enhanced with HSYA treatment along with increases in antioxidant activity and reduction of LP. HSYA also demonstrated a significant reduction in matrix metalloproteinases, which play a role in various neurological diseases. It is unclear if this natural compound will provide the same level of protection if initiated after the trauma. There does not appear to be a clear mechanism of action for this compound, other than a reduction in oxidative stress.

Lovastatin

Lovastatin is naturally occurring statin found in oyster mushrooms (*Pleurotus ostreatus*) and red yeast rice. An experimental study used a pretreatment paradigm to evaluate lovastatin as a neuroprotective against following a moderate CCI-induced TBI injury.[128] Adult rats were injected with lovastatin (4 mg/kg, i.p.) for 5 days prior to a unilateral injury centered over bregma. At 6 h post-trauma, messenger RNA (mRNA) levels for TNF-α and IL-1β were significantly reduced. At 4 days post-injury, the authors reported a significant reduction in contusion volume coupled with a reduction in FJB staining. Lovastatin also decreased the expression of proinflammatory cytokines, TNF-α, and IL-1β. An abbreviated neuroscore also showed significant improvement over the first 7 days. This natural compound has not been evaluated when therapy is initiated post-trauma. There is no TBI-related mechanism suggested for this compound other than it dampens the neuroinflammatory response.

Luteolin

Luteolin is a plant-derived flavone found in a variety of vegetables such as broccoli and celery, and has been isolated from a variety of aromatic plants, including members of the mint family. Recently, luteolin has been evaluated as a therapeutic intervention following a diffuse injury to the frontal cortex in adult mice.[129] Using a modified weight-drop closed head injury model, animals were treated with 10, 30, or 50 mg/kg of luteolin i.p. 30 min post-trauma. All doses of the compound significantly decreased edema. Both the 10 and 30 mg/kg doses significantly improved grip strength. Coupled with this was a reduction in oxidative stress and an increase in the antioxidant GPx. This natural compound was also shown to significantly decrease TUNEL staining. In a follow-up study,[130] this same group reported a significant reduction in BBB opening along with a decrease in FJB staining immediately below the impact site with a 30 mg/kg single i.p. post-trauma injection. This natural compound also increased markers for autophagy, while significantly reducing markers of neuroinflammation. A 15 day pretreatment with luteolin (20 mg/kg) has also been shown to reduce TBI-induced Alzheimer's disease (AD) pathology in a transgenic mouse model following a penetrating TBI with a CCI model.[131] The most recent study evaluated luteolin coupled with an antineuroinflammatory compound, palmitoylethanolamide (PEA).[132] Adult CD1 mice were subjected to a moderate CCI trauma and treated i.p. at 1h post-injury with either 1 mg/kg or 10 mg/kg of the combination of PEA and luteolin (PEAL). It appears that only the 1 mg/kg dose was beneficial, because results using the 10 mg/kg dose are not reported. PEAL significantly helped neuroscores, and reduced edema. Infarction identified with the triphenyltetrazolium (TTC) method was also significantly reduced, as was the overall histology score. As expected, neuroinflammation and oxidative stress was significantly attenuated. Luteolin has been linked to activation of the nuclear factor erythroid2-related factor 2 (Nrf2) and its high affinity to the antioxidant-responsive element (ARE). This Nrf2/ARE pathway activates a variety of antioxidants including GPx.[133,134]

Morphine

Morphine is an opioid (opium alkaloid) compound that can be extracted from the unripe seedpods of the poppy (*Papaver somniferum*). It is commonly used to induce sedation and calm pain in many clinical conditions including brain injuries/surgeries.[135–137] One of the earliest studies to evaluate morphine's effects following TBI used a mild midline FP injury in adult rats.[138] Animals were treated prior to TBI with 10 mg/kg i.p. of morphine, and assessed $\sim$12 min later for changes in acetylcholine turnover. Morphine prevented injury-induced changes in a variety of different subcortical structures. The first study to evaluate possible cognitive function following TBI and morphine again used a moderate midline FP injury in adult rats.[139] Animals were treated with 10 mg/kg i.p 15 min prior to injury, and subsequently tested for their beam walk ability. Morphine treatment enhanced this motor skill compared with saline treatment. A follow-up study using the same injury paradigm evaluated adult rats over a longer post-trauma time course.[140] The morphine treatment failed to show a significant improvement in either beam walking or beam balance over a 10 day evaluation. In a rather interesting study, adult male rats were subjected to a moderate CCI injury, and seven different anesthetic agents commonly used in experimental TBI studies were evaluated.[141] Animals treated immediately post-trauma with morphine (15 mg/kg, i.v.) performed very poorly on the balance beam and beam walking during the initial 5 days post-trauma. These same subjects were evaluated in the MWM and failed to show significant improvement. Histological analysis at 21 days also failed to show a beneficial effect of the morphine treatment in terms of lesion volume or hippocampal neuronal sparing. A study evaluating young adult mice subjected to a unilateral closed head weight-drop injury[142] reported significant improvement in the MWM with a 10 mg/kg i.p. treatment with morphine immediately following the trauma. This is a rather unusual set of results, because there were only long-term beneficial effects of the morphine treatment and no short-term effects. Several human studies suggest that morphine may be beneficial following head trauma.[135,136,143]

Naringin

Naringin is a citrus flavonoid that is commonly found in grapefruit and known for its strong antioxidant and antiapoptotic properties. When metabolized, it becomes naringenin which can cross the BBB. A relatively recent study evaluated naringin's neuroprotective effects following a contusion type TBI in adult

rats.[144] Some rats were pretreated for 7 days with an oral dose of naringin (100 mg/kg) and other rats were treated for an additional 7 days after trauma. Naringin treatment significantly reduced edema and also reduced motor deficits beginning at 7 days post-injury. The authors report a significant reduction in MDA levels and an upregulation of SOD with the extended 7 day treatment. Markers of neuroinflammation were also reduced. The fact that the injury was performed with a device designed for spinal cord injury is somewhat surprising. It is unclear what the actual lesion volume was for this type of effect. There was no specific mechanism suggested for these positive results.

Nicotine

Nicotine is the main alkaloid in tobacco plants (*Nicotiana tobacum*) and may also occur in some other edible plants. Nicotine has a direct action not only on nicotinic acetylcholine receptors, but also parts of the dopaminergic system. An initial study evaluated chronic infusion of nicotine via osmotic pumps following a mild or moderate CCI injury[145] in adult rats. Nicotine was given for 7 days, and subjects were evaluated for possible changes in cortical lesion volume and also α7* nicotinic acetylcholine receptors (α7* nAChrs). A dose of 0.125 mg/kg/h decreased lesion size and reversed the α7* nAChrs injury-induced deficits. A follow-up study[146] investigated whether or not post-injury therapy was equally effective as pretreatment. Adult rats were treated for an extended period of time either before the injury (0.3 mg/kg; twice daily) or for an extended period post-injury, and subsequently tested in a MWM. Although the post-injury treatment helped, the animals still showed a deficit. The nicotine failed to show any reduction in lesion volume. A more recent study demonstrated that post-injection of nicotine (2 mg/kg, i.p.) for 7 days has a significant beneficial effect on the dopaminergic system following a moderate TBI.[147] There has not been a concerted effort to identify an effective therapeutic window or a detailed dose-response curve for nicotine following TBI. Upregulation of α7* nAChrs is believed to be the underlying mechanism for the effects described.

Nitidine

Nitidine is an alkaloid isolated from the *Zanthoxylum nitidum* plant and has been identified for its potent anti-inflammatory properties in both *in vitro* and *in vivo* experiments. The zanthoxylum plant has long been used as a traditional medicine for the treatment of inflammatory diseases such as rheumatic arthritis. Recently, nitidine was evaluated for possible therapeutic efficacy against inflammation-mediated neuronal death following TBI.[148] The injury consisted of inserting a 21g needle in the right parietal cortex of young adult mice and investigating the brain for changes in microglial activation. Nitidine treatment (2.5 mg/kg; i.p.), administered immediately after injury and once daily, significantly increased neuronal survival, as shown by NeuN/TUNEL positive cells, and decreased reactive microglia in the ipsilateral cortex 3 days post-injury. This type of injury does not create any cognitive or motor deficits. The downregulation of phosphorylation of mitogen-activated protein kinases extracellular signal-regulated kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK), and also NF-kB signaling pathways, may play a significant role in nitidine's therapeutic intervention.

Puerarin

Puerarin is an isoflavone *C-glycoside* found in Chinese herbs such as *Pueraria lobata* and *Pueraria thomsoni*. This compound has been widely used in traditional remedies for the treatment of various ailments such as cardiovascular disorders and ischemic stroke. Recently, puerarin has shown to be protective in a rat model of TBI.[149] Adult rats were pretreated with puerarin (200 mg/kg; i.p.) prior to a moderate/severe injury using the Feeney TBI model.[90] This group reported a significant amelioration in MDA levels coupled with increased levels of GSH in the contused cortex at 24 h post injury. In addition, this natural compound increased the levels of myeloperoxidase while demonstrating neuroprotection by a decrease in FJB staining. Unfortunately, there was no assessment of cognition or evaluation of lesion volume. The mechanism responsible for the neuroprotection was reported to be enhanced activation of the phosphatidylinositol 3-kinase (Pl3K)-Akt pathway.

Pycnogenol (PYC)

PYC is a patented combinational bioflavonoid extracted from the bark of the French maritime pine tree, *Pinus maritima*. In experimental studies, PYC has been shown to have a significant potential to scavenge free radicals, promote cellular health, and improve cognitive performance. There are four recent studies evaluating the possible therapeutic value of PYC after head trauma. All four studies are published by the same research group. In the initial study,[150] young adult rats were subjected to a moderate unilateral CCI injury and subsequently treated immediately post-trauma with PYC (100 mg/kg, i.p.), followed by two supplemental doses at 3 h and 6 h post-injury. This natural compound significantly increased a variety of antioxidants including GSH, GPx, SOD, and catalase, while decreasing multiple markers of oxidative stress (TBARS, PC, 4-HNE, 3-NT) in both the cortex and the hippocampus. In addition, PYC spared multiple different synaptic proteins in both the cortex and the hippocampus at 96 h post-injury. Finally, two different markers of neuroinflammation (TNF-α, IL-6) were significantly reduced with the therapy. A subsequent investigation[151] evaluated a dose-response and therapeutic window for PYC. Using oxidative stress as a dependent variable, the natural compound at 10 mg/kg i.v. showed the greatest reduction following a moderate unilateral CCI injury. This concentration also had the greatest neuroprotective effects on key synaptic proteins. PYC appeared to be therapeutically significant even when initiated at 4 h post-trauma. An electrophysiological study from this group concerning PYC reported significant improvements in hippocampal slice physiology following a moderate injury and a single i.v. injection of 10 mg/kg.[152] Animals treated at 15 min post-TBI with PYC demonstrated preserved synaptic function, synaptic strength, and LTP at 7 days post-TBI compared with vehicle-treated controls. There was no mention of any specific mechanism underlying these positive effects with PYC other than its natural antioxidant characteristic. The most recent study[153] tested whether or not the positive effects of PYC resulted in possible changes in lesion volume and improved cognitive testing following TBI. Animals were treated after the trauma in a fashion identical to the group's first PYC study and subsequently evaluated in the MWM 7 days post-injury. Two different doses were evaluated (50 mg/kg; 100 mg/kg) and both failed to show any significant improvement in MWM acquisition compared with vehicle. Although the higher dose demonstrated some reduction in lesion volume, FJB staining failed to reveal any protection in the hippocampus.

Quercetin

Quercetin is a natural bioflavonoid found in many vegetables and fruits including onions, capers, grapes, dark cherries, and radish leaves. It has strong antioxidant and anti-inflammatory

properties. An early study evaluated the possible therapeutic properties of quercetin following a midline FP injury in rats.[154] Quercetin therapy (25 μmol/kg; i.p.) was initiated at 1 h post- trauma and continued for up to 3 days with injections every 12 h. Electrophysiological analysis of cortical compound action potentials demonstrated improved amplitudes with quercetin at both 24 and 72 h post-trauma. This natural compound also showed significantly reduced myeloperoxidase activity and increased GSH levels in the cortical regions closest to the injury. In a recent study, quercetin demonstrated very robust neuroprotective effects in rats following Feeney's weight-drop induced TBI.[155] Quercetin (30 mg/kg; i.p.) was administered immediately after injury and also daily for 3 consecutive days. A week after injury, animals treated with quercetin demonstrated a significant reduction in markers of oxidative stress and proinflammatory cytokines (TNF-α, IL-1β, and IL-6). There was a significant increase in several antioxidants (GPx, SOD, catalase) following quercetin therapy coupled with a significant reduction in TUNEL staining in the hippocampus. Most remarkable was the improved MWM scores at 4 weeks post-injury. It is unfortunate that this group did not follow up with a dose-response curve and assessment of quercetin's therapeutic window. Mechanistically, quercetin has been shown to enhance the PI3K/Akt signaling pathway, and thus increases BDNF levels.[156]

Resveratrol

Resveratrol (3,4,5-trihydroxystilbene) is a natural polyphenol compound present in high quantity in grapes, nuts, and red wine. It has potent antioxidant, anti-inflammatory, and antiapoptotic properties, and has been touted for its possible therapeutic potential in a variety of diseases.[157] Resveratrol has recently been identified as a possible neuroprotective therapy following brain trauma.[158] In an early study, adult rats were subjected to a moderate cortical contusion and given a single i.p. injection (100 mg/kg) immediately post-injury.[159] The authors reported that resveratrol significantly reduced TBI-induced oxidative stress and edema at 24 h post-trauma. This dose also decreased the size of the injury observed at 14 days. There was no attempt to evaluate possible motor or cognitive dysfunction. A subsequent study evaluated the possible anti-inflammatory properties of resveratrol after a mild TBI in adult mice.[160] Following a diffuse mild closed skull brain injury, mice were given two doses of resveratrol (100 mg/kg, s.c.) with the first immediately after the trauma and the second 12 h later. The number of reactive microglia was evaluated in the cortex, corpus callosum, and hippocampus. Resveratrol significantly reduced microglia in all three areas, although it is unclear exactly how the regions of interest were identified. An enzyme-linked immunosorbent assay (ELISA) revealed a significant reduction in proinflammatory cytokines IL-6 and IL-12 in the hippocampus. A well- conducted study probed whether or not resveratrol could work as a therapeutic agent following a moderate/severe TBI.[161] Adult rats were subjected to an open skull CCI injury and subsequently given resveratrol at either 10 mg/kg i.p. or 100 mg/kg i.p. immediately after the injury and again for 2 more consecutive days. The 100 mg/kg dose significantly improved performance on both beam balance and walking within the first 5 days post-trauma. Testing in the MWM revealed a significant effect for the higher dose of resveratrol. More surprising was the fact that the 100 mg/kg dose significantly reduced cortical injury volume and protected neurons in the hippocampal CA1 and CA3 regions. An interesting set of experiments, using both *in vitro* and *in vivo* preparations, explored the possible mechanism of resveratrol's neuroprotective qualities showing that following TBI there is an upregulation of glycogen synthase kinase-3β (GSK-3β) and also ROS.[162] Following a moderate CCI injury, animals were treated immediately with 100 mg/kg of resveratrol. GSK-3β is responsible in part for the opening of the mPTP, and resveratrol suppresses this and also the upregulation of ROS. Two recent studies from the same laboratory demonstrated resveratrol's ability as a possible therapeutic intervention following TBI. In an initial study,[163] adult rats were subjected to a moderate CCI injury and received the compound (100 mg/kg, i.p.) immediately and for 2 subsequent days. Resveratrol significantly reduced edema, enhanced motor and coordination behavior, and enhanced the rat's ability to navigate a MWM. This group showed a significant protection of neurons in the hippocampus coupled with a decrease in markers of neuroinflammation. Resveratrol also significantly suppressed the expression of the TLR4, which is believed to be a mediator of autophagy and neuroinflammation. In a follow-up study,[164] adult rats were subjected to a mild/moderate weight-drop injury using the Marmarou model.[109] Animals received resveratrol (100 mg/kg, i.p.) once daily for 5 days. As with the previous study, there was a significant reduction in edema, enhanced motor and coordination behavior, and significant enhancement of MWM behavior. This natural compound showed a sparing of two synaptic proteins (synaptophysin, postsynaptic density protein 95 [PSD-95]) in the hippocampus coupled with a decrease in the expression of the microtubule-associated proteins LC3-II and Beclin that are markers of autophagy. It will now be important to demonstrate the therapeutic window and the optimal dose of resveratrol. This compound may also be working by suppressing the activation of NF-κB and the sirtulin SIRT1 pathways. These pathways are involved in the upregulation of several neuroinflammatory markers such as NO, TNF-α and IL-1β.[165,166]

Rutin

Rutin, also known as rutoside, quercetin-3-O-rutinoside and sophorin, is a flavonol glycoside found in a variety of plants and fruits, such as buckwheat, asparagus, mulberries, and citrus fruits. It is often used in various foods and cosmetic products as a preservative and to provide natural color. Rutin is also known to have diverse pharmacological properties including antioxidant, anti-inflammatory, and neuroprotective effects. It has also been shown to inhibit free radical generation and increase antioxidant enzyme activity. In a very unusual experiment, rutin therapy was delayed for 14 days following a severe injury.[167] Adult rats were subjected to a severe Marmarou weight-drop TBI[109] and allowed to recover for 2 weeks before the start of daily oral administration of rutin (20, 40, or 80 mg/kg) that continued for an additional 2 weeks. The therapeutic effects of both the 40 mg/kg and 80 mg/kg doses were quite spectacular. The improvements in cognition were almost at sham injury levels. This natural compound significantly decreased lipid peroxidation, nitric oxide, and neuroinflammation in the cortex and hippocampus to remarkably low levels. There were also significant increases in markers of antioxidants. It is unfortunate that evaluation of diffuse axonal injury, typical with this type of injury model, was not investigated. Clearly this compound awaits further investigation. No clear mechanism for rutin's very robust effects have been suggested.

Salvianolic acid B (SalB)

The dried root of *Salvia miltiorrhiza*, danshen, is one of the Chinese medicinal herbs used for the treatment of cardiovascular diseases. SalB is the most abundant active component found in

danshen extract. It possesses antioxidant and anti-inflammatory properties relevant to a variety of different disease models. A recent study evaluated SalB as a therapeutic intervention following a moderate CCI injury in adult mice.[168] Animals were treated with a single tail vein injection of SalB (25 mg/kg) at 2 h post-injury. At 24 h post-injury there was a significant reduction in brain edema coupled with a significant decline in markers of neuroinflammation. Motor function was significantly enhanced, and the results of learning in the MWM were outstanding and accompanied by a significant reduction in cortical lesion volume at day 15 post- injury. It remains to be investigated if the results could be further enhanced with a different dose. A possible mechanism may be SalB's ability to significantly reduce endothelial permeability through the suppression of TNF-α.

Triptolide

Triptolide is a diterpene triepoxide obtained from the Chinese herb, *Tripterygium wilfordii* hook, and has been a part of traditional Chinese medicine to treat fever and routine inflammatory and immune disorders. The vine is extremely toxic, but the root pulp appears to have medicinal properties. It has been shown to inhibit a variety of cytokines such as IL-1β and TNF-α. Other studies have shown that it interferes with transcription factors such as NF-kB. A formulation of triptolide, Minnelide, is currently used to treat some types of cancer. A relatively recent study[169] described the treatment of young adult rats with a single injection of various concentrations of triptolide (0.125–1.0 mg/kg) immediately following a mild CCI injury. Animals were evaluated at 1 and 3 days post-trauma and demonstrated a dose-response reduction in lesion volume. Triptolide also significantly reduced edema, TUNEL staining, and neuroinflammation. An additional group of animals treated with triptolide demonstrated increased motor function that extended out to 28 days post-trauma. Because of its ability to inhibit inflammation, it has been suggested that triptolide's neuroprotective properties may be related to suppression of the mitogen-activated protein kinase pathway.

Wogonin

Wogonin (57-dihydroxy-8-methoxyflavone), is one of the major flavonoids that occurs in the root of the Chinese herb Baikal skullcap (*Scutellaria baicalensis* Georgi). It has been historically used in treating allergic and inflammatory diseases. Wogonin has been shown to be neuroprotective in a model of global ischemia in which it attenuated hippocampal neuronal death, microglial activation, and behavioral deficits.[170] In an attempt to explore the possible neuroprotective effects of wogonin on TBI, young adult mice were subjected to a severe CCI injury and given a single dose (20, 40, or 80 mg/kg) i.p. immediately after the trauma.[171] This therapy improved long-term histological and functional outcomes. As with many other natural compounds, wogonin significantly reduced lesion volume and improved TBI-related neurological deficits. This natural compound reduced brain edema, BBB permeability, and markers of neuroinflammation (IL-1β, IL-6, NF-kB) and macrophage inflammatory protein (MCP-1). The authors also reported a significant reduction in TRL4 expression. Wogonin at 40 mg/kg showed significant neuroprotective qualities as evidenced by a significant decrease in FJB staining and TUNEL staining in the cortex. Subsequent studies should explore the therapeutic window for this promising compound and other cognitive tests. The mechanism underlying these robust therapeutic effects is similar to that of baicalein and involves the inhibition of TRL4 and NF-κB.

Natural Compounds' Safety and Selection

Most natural compounds come from plants, and often the exact part of the plant can vary tremendously; for example, flowers, leaves, or even the bark of a tree. In many cases, such natural compounds are used simply because they were part of traditional therapy. Folklore has been the evidence supporting their use for hundreds of years. Phytochemicals and other natural compounds are not necessarily safe, and the ''over the counter'' variety often contains only a very small amount of the actual ingredient. Most formulations of natural compounds are not ''pure,'' and consist of a variety of different ''ingredients.'' In many of the studies evaluated in this review, the source of the compound is not described in the published article. Even when a source is cited (e.g., Sigma), the product number indicating the grade and formulation are not included. As with any type of pharmaceutical, if an inappropriate dose/formulation is used, as well as the lack of knowledge of possible pharmacological interactions, a particular compound can cause adverse effects. For example, a large number of cases of adverse effects have been reported with dietary supplements that contain a natural alkaloid ephedra,[172,173] possibly because of unknown drug-nutrient and/or pharmacological interactions. Different components of the same compound can act differently and potentiate or negate the effects of each other when used in combination with other medications. Because of varying amounts of the active component, identical dietary supplements/compounds can have varying treatment effects. Different polyphenols extracted from *S. baicalensis* (wogonin, baicalein, and baicalein) have very diverse effects. Two different grape seed extracts have been shown to have very distinct neuroprotective activity.[174,175] Some natural compounds can have different effects *in vitro* than *in vivo.* For example, wogonin has toxic effects in *in vitro*,[174] and is neuroprotective when administered in *in vivo* studies.[171]

Natural Compounds' Access to the Brain

The diverse chemical structure of many natural compounds raises concerns as to whether or not they can cross the BBB and have a direct action on the brain. Several experimental studies and reviews have shown that natural compounds can modulate brain function, and have neuroprotective ability in different types of neurological problems.[15–18,176,177] This is a particularly important characteristic when compounds are investigated as a pretreatment. For many TBI animal models, the BBB is breached, allowing easy access for a post-trauma therapeutic intervention. Several studies have used *in situ* models of the BBB[178–181] or animal studies[182] to demonstrate a particular compound's ability to cross the BBB.

Treatment Effects after TBI

Most of the investigations evaluating phytochemical therapies reviewed report a significant ability to modulate the magnitude of multiple aspects of the secondary injury (Table 1). With few exceptions, there is always a significant suppression of either oxidative stress or neuroinflammation. In a few studies, both oxidative stress and inflammation have been assessed, and the natural compound significantly reduced both components. These changes are usually coupled with decreases in brain edema and neuroprotection as evidenced by significant declines in TUNEL staining and FJB staining. Exceptions to the declines in FJB and TUNEL staining occurred with caffeine, colchicine, and ginseng. Neuroprotection is often examined by assessment showing significant declines in lesion volume. Most studies evaluating lesion volume reported a

significant decline. It is rare to find a report indicating that a specific natural compound does not reduce lesion volume. Studies using apocynin, caffeine, colchicine, crocin, 7,8-DHF, and ginseng are the exceptions, and report no reduction or a very modest decline in the injury. Reduction in edema is a relatively common variable, with most natural compounds reporting beneficial effects. Notable exceptions were observed with some investigations of caffeine, cocaine, crocin, 7,8-DFH, ginseng, and morphine. When subjects were tested in the MWM, all phytochemical compounds were reported to improve performance, with the exception of studies using caffeine, cocaine, 7,8-DHF, morphine, and pycnogenol. These same exceptions also failed to show any significant improvement in various motor/coordination behaviors evaluated as part of a neuroscore.

Mechanism Responsible for Beneficial Effects Post-TBI

For more than half the natural compounds evaluated in the current review, the mechanism of action was stated as either free radical scavenger or ability to reduce neuroinflammation through the suppression of key kinases such as TNF-α and IL-1β. There was no indication of how these compounds were able to accomplish this. A number of studies have suggested that the phytochemical under consideration achieved its therapeutic benefit by inhibition of a particular pathway. For example, apocynin is a known inhibitor of NADPH oxidase, and this mechanism can significantly reduce oxidative stress. A number of the agents reviewed inhibit TLR4 and NF-κB (baicalein, curcumin, nitidine, resveratrol, wogonin). Studies involving other compounds cite activation of specific pathways such as Akt/eNos (allicin, colchicine), Notch signaling (crocin), TrkB/Akt (7,8-DHF), PI3K/Akt (puerarin, quercetin), and Nrf2-ARE (luteolin). It is currently unclear whether or not these specific pathways are responsible for the diversity of effects attributed to a particular natural compound. In some cases, it has only been suggested that a particular mechanism may be responsible following TBI based on its actions following other type of neurological problems such as ischemia. These studies support the idea that phytochemicals are dynamic compounds capable of significant CNS alterations.

Methodological Discrepancies

Of the 33 different compounds reviewed, 17 have only been evaluated once, and half of these were administered as a pretreatment only. Although much can be learned from pre-injury treatment, post-trauma therapeutics are the aim of pharmacological intervention. Two variables that significantly complicate interpretation are the dose of the pharmaceutical and the duration of the therapy. Approximately half of the studies evaluating phytochemicals gave a single post-injury injection of a particular compound. Many studies gave multiple doses ranging from a few hours to a week post-injury. Of the 33 different natural compounds evaluated, only 12 were investigated using a dose-response paradigm that included at least three different levels of the compound.[63,69,78,80,83,91,101,126,127,129,151,167,171,183] Most studies simply used a dose taken from the literature, possibly unrelated to brain injury. Often doses are arbitrarily altered depending upon whether the subject is an adult rat or mouse.

The type of injury model is extremely important, because different models produce dramatically different histological outcomes. For example, there are fundamental differences in the histopathology associated with different weight-drop models of TBI, and very often researchers inappropriately equate injuries produced with the Marmarou, Feeney, and Shohami models.[90,109,184,185] There are multiple excellent reviews that have detailed not only the histopathology associated with each model but also the type of cognitive and behavioral changes associated with each.[186–191] Injury severity is also extremely important in determining the potential therapeutic value of a compound. In most of the studies evaluated in this review, the injury severity is not clearly stated, and no reference is provided that the particular injury model can produce a range of severities. A pharmaceutical applied following a severe cortical contusion will most likely not produce the same beneficial effect compared with a mild or moderate injury. If a compound is reported to help with a mild TBI, one needs to know how that level of injury is actually evaluated. Unless the reader is aware of the magnitude of the histopathology, or representations of the injury are provided, it is difficult to evaluate the results. Subtle differences in the placement of an injury can dramatically alter the histopathology and behavioral outcome.[192,193] Post-injury evaluation of dependent variables is critical to proper assessment of therapeutic outcome.

Some measures of SIC have time and methodological constraints to properly evaluate pharmacological effect. For example, edema needs to be assessed within 24–48 h post-trauma. Neuronal degeneration as evaluated by FJB has a critical time-dependent assessment window of 24–48 h.[194] Evaluation of many aspects of a neuroscore, which include measures of motor deficits such as beam balance, rotarod, and grip strength, is also time dependent, because many of these behaviors spontaneously recover over time. Cognitive tests such as the MWM must allow subjects to recover from the surgical procedures before they can be properly tested. Finally, assessment of overall neuronal loss and changes in injury volume should be performed in an unbiased fashion using routine stereological methods.[195–198]

Conclusions

There are a large number of phytochemicals that claim to protect or enhance the outcome following experimental TBI. With few exceptions, almost all of these natural compounds show significant modulation of secondary injury cascades. It is difficult to publish so called ''negative'' results; however, these are very important. For almost every phytochemical reviewed, there is at least one study, with the exception of cocaine, colchicine, morphine, and nicotine, that has reported a reduction in either oxidative stress or neuroinflammation. No clear positive effects following experimental TBI have been reported for any of these four ''exception'' compounds. Presently, the evidence to support the use of phytochemicals following this type of brain injury is tenuous at best. Very few, if any, studies have performed a complete evaluation of the primary components of SIC. Resveratrol has been the most thoroughly evaluated as a post-trauma therapeutic agent, and has been shown to modulate multiple aspects of SIC. Studies from two different laboratories have reported significant cognitive enhancement when this compound was given for an extended period of time post-injury.[161,163,164] The severity of injury was at a moderate level using the same injury model. Other laboratories have reported resveratrol's capacity to decrease oxidative stress and neuroinflammation as a post-injury therapy.[162,163] Although it is unfortunate that no one has reported a decline in FJB or TUNEL staining with resveratrol, it has been reported to reduce edema and lesion volume, and to spare hippocampal neurons and key synaptic proteins.[159,161,163,164] Clearly, more work needs to be done in a systematic manner to clarify if a specific compound is capable of enhancing a favorable outcome following brain injury.

The researcher interested in working with natural compounds as a therapeutic intervention following TBI should be very cautious in accepting some of the evidence presented in the literature. Often, if the results sound simply too fantastic to be true, they just may not be. The beneficial effects of natural compounds in the field of stroke and ischemia are very substantial, and have been the impetus for many of the TBI studies reviewed here. Perhaps with well-controlled and properly executed studies in TBI, this literature will also evolve. The overall literature is very promising, and it is to be hoped that natural compound therapies may eventually play an important role in the pharmacological management following TBI.

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