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The Role of Reactive Oxygen Species in Traumatic Brain Injury

Experimental Studies in the Rat

BY

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ABSTRACT

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Traumatic brain injury (TBI) is a major cause of mortality and disability. As common sequelae in survivors of TBI are disabling functional, emotional and cognitive disturbances, improved treatment of TBI patients is urgently needed. At present, no neuroprotective pharmacological treatment exists. The formation of oxygen-centered free radicals, reactive oxygen species (ROS), is considered an important event in the pathophysiology of TBI. In the present thesis, the fluid percussion (FPI) and controlled cortical contusion injury models of TBI in rats were used. Two nitrone radical scavengers, α-Phenyl-N- *tert* -butyl nitrone (PBN) and the sulfonated analogue of PBN, 2-sulfophenyl-N-*tert*-butyl nitrone (S-PBN), were used as tools to study the role of ROS in TBI.

Pre-treatment with PBN (30 mg/kg) improved morphological and cognitive outcome after severe controlled cortical contusion injury. Treatment with equimolar doses of PBN and S-PBN administered 30 min after FPI followed by a 24 h intravenous infusion improved morphological outcome. Only S-PBN improved cognitive outcome as assessed in the Morris Water Maze. Surprisingly, pre-treatment with PBN increased the number of apoptotic neurons at 24 hours after injury despite a reduced lesion volume. FPI resulted in an early increase in glucose uptake and a reduction in regional cerebral blood flow (rCBF) assessed by fluoro-2-deoxyglucose (FDG) and hexamethylpropylene amine oxime (HMPAO) autoradiography. At 12 h, a marked reduction in glucose uptake and rCBF ensued. These TBI-induced changes were attenuated by PBN and S-PBN pre-treatment.

A method for ROS detection using 4-hydroxybenzoate in conjunction with microdialysis was evaluated. The results showed a marked increase in ROS formation as assessed by an increase in the single adduct 3,4-DHBA, lasting 90 min after injury. In a separate study, PBN and S-PBN equally reduced 3,4-DHBA formation despite no detectable brain concentrations of S-PBN at 30 or 60 min post-injury.

In conclusion, ROS play an important role in the injury process after TBI. We report a method for ROS detection with potential clinical utility. Nitrones increased ROS elimination and improved functional and morphological outcome. Nitrone treatment may have a clinical potential as a neuroprotective concept in TBI.

Key words: Apoptosis, CBF, controlled cortical contusion injury, 3,4-dihydroxybenzoate, fluid percussion injury, glucose, 4-hydroxybenzoate, microdialysis, Morris Water Maze, neuroprotection, nitrones, PBN, rat, reactive oxygen species (ROS), salicylate, S-PBN, TBI.
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To Pia, Elin, Oscar

Framtiden kommer av sig själv,
framsteg gör det inte.

P. Henningsen

ARTICLES INCLUDED

This thesis is based on the following articles, which will be referred to by their Roman Numerals. Reprints were made with the permission of the publishers;

I Marklund N, Clausen F, Lewén A, Hovda D.A, Olsson Y and Hillered L (2001) α-Phenyl- *tert* -N-butyl nitrone (PBN) improves functional and morphological outcome after cortical contusion injury in the rat. *Acta Neurochirurgica* 143: 73-81

II Marklund N, Clausen F, McIntosh TK, Hillered L (2001) Free radical scavenger post-treatment improves functional and morphological outcome after fluid percussion injury in the rat. *J Neurotrauma* In Press

III Lewén A, Skoglösa Y, Clausen F, Marklund N, Chan PH, Lindholm D, Hillered L (2001) Paradoxical increase in neuronal DNA fragmentation after neuroprotective free radical scavenger treatment in experimental traumatic brain injury. *J Cereb Blood Flow Metab* In press

IV Marklund N, Sihver S, Långström B, Bergström M, Hillered L (2001) Effect of traumatic brain injury and nitrone radical scavengers on changes in regional cerebral blood flow and glucose uptake in rats. Submitted

V Marklund N, Clausen F, Lewander T, Persson L and Hillered L (2001) Monitoring of reactive oxygen species production after traumatic brain injury in rats with microdialysis and the 4-hydroxybenzoic acid trapping method. Submitted

VI Marklund N, Lewander T , Clausen F, and Hillered L (2001) Effects of nitrone radical scavengers on *in vivo* trapping of reactive oxygen species after traumatic brain injury in rats. Submitted

Abbreviations used in the present thesis

ATP Adenosine triphosphate
BBB Blood brain barrier
CNS Central nervous system
DAI Diffuse axonal injury
3,4-DHBA 3,4-dihydroxybenzoic acid
ECF Extracellular fluid
FDG [^{18}F] Fluoro-2-deoxyglucose
FPI Fluid percussion injury
2-HBA 2-hydroxybenzoic acid; salicylate
4-HBA 4-hydroxybenzoic acid
[^{99m}Tc]HMPAO 99mTc-Hexamethylpropylene amine oxime
HPLC High performance liquid chromatography
ICP Intracranial pressure
i.p. intraperitoneal
i.v. intravenous
MD Microdialysis
NO Nitric oxide
NADP Nicotinamide dinucleotide phosphate
PARP Poly(ADP-ribose) polymerase
PBN α-phenyl-N-*tert* butyl-nitrone
PMN Polymorphonuclear neutrophils
rCBF Regional cerebral blood flow
ROI Region of interest
ROS Reactive oxygen species
SOD Superoxide dismutase
S-PBN 2-sulfophenyl-N-*tert*-butyl nitrone
TBI Traumatic brain injury
TUNEL Terminal deoxynuclotidyl transferase –(TdT) mediated biotinylated dUTP nick end labeling
WBC White blood cells

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[If thou examinest a man having a gaping wound in] his [head], penetrating to the bone, (and) perforating his skull; thou shouldst palpate his wound; [shouldst thou find him unable to look at his two shoulders] and his breast, (and) suffering with stiffness in his neck…
Diagnosis; Thou shouldst say [regarding] him: " One having [a gaping wound in his head, penetrating to the bone, (and) per]forating his skull, while he suffers with stiffness in his neck. An ailment which I will treat."
Treatment: Now [after thou has stitched it, thou shouldst lay] fresh [meat] upon his wound the first day. Thou shouldst not bind it. Moor (him) [at his mooring stakes until the period of his injury passes by]. Thou shouldst [tre]at it afterwards with grease, honey and lint every day, until he recovers….
From *The Edwin Smith Surgical Papyrus,* dating from the seventeenth century BC[338]. Also see *www.neurosurgery.org/cybermuseum/*

BACKGROUND

Traumatic brain injury (TBI) is a devastating disease. TBI most commonly results from motor vehicle accidents, assaults and falls and is often associated with damage to other organ systems. TBI is the third leading cause of death in the Western Wold and affects twice as many men as women. Because the incidence for TBI peaks in youth, the TBI-related loss of working year precedes cancer and cardiovascular disease[267]. In Scandinavia, approximately 200/100,000 inhabitants are hospitalized for TBI annually[280]. It has been estimated that 75-90 % of all head injuries are of mild or moderate severity[188], with surgical intervention performed in up to 4% of patients. The outcome after brain injury is related to the severity of the injury; early prognostic factors include early Glasgow Coma Scale (GCS) score, pupillary reactivity and age[197]. The modern era of TBI management and neurointensive care was started in the 1960´s through the pioneering work of Lundberg in Sweden, who introduced the measurement of intracranial pressure (ICP) through a catheter in the lateral ventricles of the brain[210]. Subsequently, it was found that increased ICP correlated with poor outcome[15;220]. ICP monitoring is now routinely performed in many neurocritical care centers worldwide. In Glasgow, observations were made that many of the patients ultimately dying from TBI had been able to talk on admission to the emergency room ("talk and die" cases[220;236]). It was concluded that the primary damage in these patients was mild and that the subsequent deterioration was due to secondary injury factors that theoretically would have been possible to prevent, i.e. "avoidable factors", such as

hypoxemia, hypotension, seizures, increased ICP and hyperthermia[80;278]. Since then, there has been rapid improvements in the management of severely injured TBI patients that has resulted in an improved outcome[332]. The improved outcome has mainly emerged from better monitoring and surveillance through the introduction of specialized neurointensive care units (NICU) and aggressive treatment of potentially avoidable secondary injury factors. The knowledge of the pathobiology of human TBI is rapidly expanding through the introduction of new monitoring modalities, including intracerebral microdialysis, continuous EEG monitoring, jugular bulb oxygenation, and brain tissue oxygen monitoring[14;174;259;327;328;329].

Common sequelae for survivors of TBI, are impairment of information processing, perceptual function and memory[25;196]. Additionally, irritability, immature social behavior, diminished motivation, depression and personality changes commonly persist[36;163;196]. Approximately 15 % of patients with mild TBI have disabling symptoms one year after the injury[231]. These results emphasize the importance of evaluating functional and cognitive outcome in addition to morphological outcome in experimental and clinical TBI research. Consequently, further improvements in the management of TBI victims are urgently needed. Until recently, a number of clinical trials with neuroprotective compounds with promising pre-clinical documentation have all failed to demonstrate convincing efficacy in a broad population of head-injured patients[71]. Currently, no pharmacological treatment for TBI with proven efficacy exists. Vast experimental evidence suggests that a pharmaceutical compound may be given within a clinically relevant time-window[225] and hence the search for neuroprotective compounds with clinical application continues.

The aim of the present thesis is to evaluate the role of reactive oxygen species (ROS) in experimental TBI. The nitrones α-phenyl-N-*tert* butyl nitrone (PBN) and the sulfonated analogue of PBN, 2-sulfo-phenyl-N-*tert*-butyl nitrone (S-PBN) are well-known spin trapping agents and are used as tools to study ROS involvement in the injury process and outcome of TBI. Additionally, a method for *in vivo* ROS detection is evaluated.

PATHOPHYSIOLOGY OF TBI

Biomechanics and classification

Brain injury (e.g., dysfunction and structural failure of brain tissue) is caused by the relative motions and displacement generated within the brain resulting from external forces (either contact or acceleration/deceleration) acting on the brain[104;112;312]. There are two types of motion that are important in the pathophysiology of TBI: translational (i.e. where the head accelerates along a straight path) and rotational (i.e. rotation of the head, causing large motions of intracranial content and tissue strains). The majority of human TBI are closed head injuries[122] and typically classified into two categories. (a) focal brain damage, including epi- and subdural hematomas, contusions and intracerebral hematomas or (b) diffuse brain damage, including axonal injury (DAI)[227]. DAI often results from rotation of the head and occurs in approximately 30 % of TBI[2;151]. DAI is commonly associated with severe neurological deficits and poor outcomes (e.g., a persistent vegetative state). It was originally considered irreversible at the time of injury; this view, however, has been challenged by experimental reports showing axonal recovery after injury[37;250]. Neural injury has been well documented in the first few hours after human TBI in regions such as the cerebral cortex, hippocampus, thalamus and substantia nigra[186]. Cortical contusions are most commonly located in the frontal and temporal regions and may be classified as coup (originating at the site of impact) or contrecoup (contralateral to the site of injury). Some persisting common neuropsychiatric symptoms may be explained by focal injury to certain brain regions. For instance, orbitofrontal contusions are associated with posttraumatic irritability and disinhibited behavior[60] and anterior left hemispheric lesions are associated with depression post-injury[277]. Although most research has focused on neuronal injury, axonal, glial and vascular injury occurs simultaneously[245;261]. The impact to the brain, be it either contact or acceleration/deceleration forces, sets in motion a highly complex cascade of secondary neurochemical events exacerbating the injury. Some major events related to the pathobiology of TBI are outlined in the next section.

Important physiological and neurochemical events

Considerable experimental evidence suggests that the morphological and functional outcome only in part reflect the primary, mechanical insult and the secondary neurodegeneration observed after TBI is continuing up to several years after injury[31;304]. The initial impact causes a depolarization and disturbance of membrane ion homeostasis with potassium efflux and calcium (Ca^{2+}) influx[166;246;248]. Increases in intracellular Ca^{2+} occur after TBI, persisting up to 4 days after the injury[87;167;340]. The Ca^{2+} influx is believed a key event in TBI, causing mitochondrial damage, an increase in free radical production, changes in gene expression and activation of numerous enzyme systems with cell-destructive properties such as calpain, caspases and phospholipases[226].

The excessive release of excitatory amino acids (EAA), particularly glutamate, from intracellular pre-synaptic vesicles into the extracellular space[352], is an important cause for neuronal loss after ischemia and trauma in the CNS. Under normal circumstances, the levels of glutamate are tightly regulated by energy dependent uptake by astrocytes[89]. Excessive release of EAA causes excitotoxicity from overstimulation of glutamate receptors such as the N-methyl-D-aspartate (NMDA)-receptors. Activation of glutamate receptors causes Ca^{2+} and sodium influx with a concomitant passive influx of water and chloride ions resulting in cell swelling[352]. Excessive interstitial levels of glutamate are commonly observed in experimental and human TBI[84;247;259].

Impairment of mitochondria, the powerhouse of the cells, appears to be an important event after TBI. Mitochondria may be a primary target for excitotoxicity[89] and an increase in intracellular Ca^{2+} causes mitochondrial disturbance with inhibition of oxidative phosphorylation[75;287;340]. Impairment in mitochondrial electron transfer and energy transduction was shown to last from 1 h to a minimum of 14 days after TBI in rats[340]. The impairment of mitochondrial function results in a decreased capacity for ATP production, occurring at a time after injury with increased energy demand for restoring ion disturbances and cell repair.

The blood-brain barrier (BBB) provides numerous important functions for the brain, including protection of the brain from toxic substances derived from the blood. Astrocytes induce BBB properties in brain endothelial cells including the formation of

tight junctions[178]. TBI imposes a biphasic disturbance of the BBB allowing passage of substances normally confined to the blood stream[141] with important implications for the penetration of drugs used in the management of TBI patients.

Inflammation with the production of cytokines (e.g., TNF-α, IL-1, IL-1β and IL-6), infiltration of neutrophils into brain tissue and upregulation of adhesion molecules is increasingly recognized as an important secondary event in TBI[225]. The precise role of inflammation in TBI is not evident, although it has several adverse effects on traumatized tissue such as reduction of blood flow through obstruction of microvessels and production of reactive oxygen species (*vide infra*). Infiltration and accumulation of polymorphonuclear neutrophils (PMN) into brain parenchyma post-TBI start at 4-6 h after injury, peaks at 1-2 days post-injury[307], and is associated with brain swelling[152;289]. Inhibition of PMN accumulation improved neurobehavioral recovery after TBI[165]. Release of cytokines after injury has been linked to activation of astrocytes[311;282], ultimately producing scar tissue, astrogliosis, that may inhibit the repair process[13]. In addition, leukocyte infiltration and cytokine production is found in human TBI[59;153].

Numerous other secondary injury factors have been implicated in the cascade aggravating the initial damage after TBI. Some of these secondary injury factors include neurotrophins, phosholipid degradation, ion disturbances and changes in acetylcholine release and metabolism (for a review see McIntosh[225]). An exciting aspect of experimental TBI in recent years is the discovery of neural stem cells[52]; their importance in the pathophysiology of and the treatment for TBI will likely increase during the next decade of TBI research.

CEREBRAL BLOOD FLOW AND GLUCOSE METABOLISM

Both hemodynamic and metabolic factors have been implicated in the complex pathobiology of TBI[109]. Within wide variations in cerebral perfusion pressure, cerebral autoregulation ensures a steady blood supply to the brain by adjusting cerebrovascular resistance[256]. Numerous chemical and physical factors have been shown to contribute to regulation of vascular tone. Regional cerebral blood flow (rCBF) is mainly

regulated by changes in the resistance of cerebral arteries, the larger cerebral arteries comprising approximately 45-50% of the total resistance (for a review see Golding[111]). The brain receives about 15% of the cardiac output. When CBF is reduced below 30% of normal values, action potentials can no longer be generated and brain electrical activity ceases[33]. Cerebral ischemia may be defined as the level of blood flow at which the brain has reached maximum oxygen extraction capacity and at which the cerebral metabolic rate of oxygen ($CMRO_2$) becomes directly dependent on CBF[276]. Classically, cerebral ischemia has been defined as a blood flow of less than 18 ml/100g/min[29]. Ischemic brain damage is one of the most important mechanisms underlying secondary brain damage in TBI[235], occurring at an early stage in at least 30% of severely head injured patients[29;290]. In patients dying from severe TBI[118], 90% had ischemic lesions at autopsy, most commonly seen in the hippocampus and basal ganglia[186]. The reasons for the decreased CBF after TBI are not clear, although cerebral vasospasm has been observed angiographically or with transcranial Doppler after severe human TBI[221;279]. After experimental TBI, cerebrovascular disturbance may be caused by microvascular obstruction from platelet and WBC accumulation[70;144] and is commonly manifested as an increase in BBB permeability and cerebral edema[176;314], resulting in impairments of autoregulation and vascular reactivity[240].

In the normal *in vivo* state, glucose is virtually the only substrate for energy metabolism in the brain; an uninterrupted delivery of glucose and oxygen is crucial for normal brain function. Hyperglycolysis, defined as an increase in glucose utilization relative to oxygen metabolism, is a common finding across animal models early after TBI using the deoxyglucose method[7;155;168]. The increase in glucose metabolism post-injury may be a response to restore ion disturbances or the repair of cell damage through activation of highly energy-consuming repair enzymes[49;335]. To meet the need for additional ATP, an increase in glycolysis is necessary as oxidative capacity normally runs at peak capacity[270;281]. The combination of increased glycolysis and impaired mitochondrial function may in part explain the observed elevation of extracellular lactate commonly seen after human and experimental TBI[170;189;247;260].

Increased levels of extracellular glutamate may induce hyperglycolysis as administration of glutamate receptor antagonists was shown to attenuate the increased glucose metabolism after fluid percussion injury[162;169] and extracellular infusion of glutamate induced hyperglycolysis[98]. Furthermore, an increased activity of glucose transporter protein 1 was demonstrated near contusions in severely brain injured patients[57]. Finally, WBCs may contribute to the increase in hyperglycolysis through the release of interleukin 1-β and TNF-α that may induce an increase in astrocytic glucose metabolism[349].

In severe human TBI[23], global hyperglycolysis or hyperglycolysis adjacent to focal brain lesions has been observed with a longer duration (up to 5-7 days) than in the experimental setting[7;23;154]. Similarly, the increase in extracellular glutamate persists for several days in the human brain[38;114;187;260] but is short-lived in rodent models of TBI[247]. At a later stage after injury, glucose uptake was reduced up to 30 days after human brain injury[22], and decreased metabolic rates of glucose in medial temporal, posterior temporal and posterior frontal cortices at 3-12 months after injury were shown to correlate with deficits in attention and memory[156]. Similarly, the early increase in glucose uptake is followed by a prolonged period with decreased glucose uptake in rodent models of TBI, starting at 1-4 h post-injury[67].

MODES OF CELL DEATH

An undesired endpoint in many disorders of the nervous system, including TBI, is the death of neural and non-neuronal cells (glia, endothelial cells, etc.). The two major types of cell death, necrosis and apoptosis, are often defined by morphological criteria[173;339]. Apoptosis has been considered the morphological manifestation of programmed cell death that occurs during development[205] and is the end result of a strictly regulated genetic program that requires ATP and protein synthesis for its execution[294;339]. In apoptosis, there is a series of typical nuclear changes, including chromatin condensation and margination with DNA fragmentation resulting in DNA laddering that can be detected by TUNEL staining in nuclei[103;331]. One important discriminant between apoptosis and necrosis is the implication the death of an individual cell or a group of cells has to its neighbors. In apoptosis, cell death is

induced with minimal release of genetic material and other pro-inflammatory intracellular constituents[352] whereas necrosis is considered a passive event with cellular breakdown and spilling of intracellular glutamate and lyzosomal enzymes that cause inflammation and damage to neighboring cells.
Apoptotic cells are rapidly and efficiently sequestered by phagocytes before lysing[195] and are found in scattered cells rather than in foci. Chromatin condensation, nuclear fragmentation, decrease in cellular volume, membrane blebbing and nuclear fragmentation are seen as morphological manifestations of apoptosis. In necrosis, mitochondria appear swollen and cells display vacuolated cytoplasm and pycnotic nuclei[274;314].

Biochemically, apoptosis may be divided into extrinsic and intrinsic arms. The intrinsic arm originates in the mitochondria with the release of cytochrome c through the mitochondrial permeability transition (MPT); the intracellular cytochrome c then causes the formation of the "apoptosome complex" (Apaf-1, dATP and caspase-9 zymogen)[45], which in turn activates a family of cystein proteases known as caspases[3;173;303]. Caspases play a crucial role in mediating apoptosis, probably by cleaving multiple cellular proteins ultimately causing cell death[321]. The extrinsic arm including the formation of a caspase activating complex is initiated by the binding of "death factors" such as Fas ligand to cell surface receptors belonging to the TNF/nerve growth factor receptor superfamily[35;244]. One important effector (executioner, down-stream) caspase is activated caspase-3[77] that was analyzed in the present thesis as an indicator of ongoing biochemical apoptosis. Neuronal death *in vivo* is heterogeneous and exhibits morphological characteristics that may represent a continuum between necrosis and apoptosis[211;268]. Traditionally, cell death after TBI was associated with necrosis[68], a view challenged when apoptosis was observed in TBI models[54;55]. It is now clear that after TBI, both apoptosis and necrosis are observed[54;55], affecting astrocytes and oligodendroglial cells as well as neurons[245].

REACTIVE OXYGEN SPECIES (ROS)

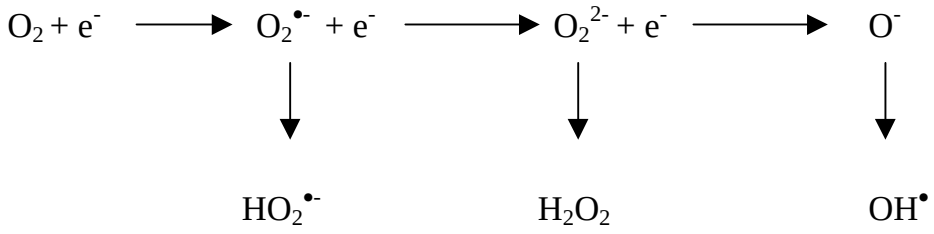
Love is like oxygen; you get too much - you get too high,
Not enough and you're going to die

The Sweet (Level Headed, 1978)

Chemistry of ROS

Under normal conditions, atoms or molecules are surrounded by electron orbitals containing two spin paired electrons per orbital. The definition of a radical is any atom or group of atoms that has an unpaired electron in the outer orbital, that is capable of independent existence. The unpaired electron makes the radical highly reactive; when the radicals react with other compounds, they may either donate the unpaired electron (i.e. reduction) or extract an electron (i.e. oxidation), in turn producing new radicals. To obtain energetic stability, a companion electron is attracted at the expense of a neighboring molecule. When two radicals react, both radicals are eliminated as radicals, but when a radical reacts with a non-radical, another radical is formed. Because most molecules present in living organisms are non-radicals, a reaction with a free radical will most likely create a new radical.

Oxygen, now an abundant element in the biosphere, first began to appear 2-3 million years ago[133] and is the quintessence for biological life. However, oxygen excess is toxic; in 1954, oxygen toxicity was proposed to be due to oxygen radicals[105]. The oxygen molecule is in itself a weak biradical in that it has two electrons with antiparallel spins, each occupying one orbital[134;301]. Upon subsequent addition of electrons to oxygen the superoxide anion ($O_2^{\bullet -}$) hydroperoxyl radical ($HO_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$) are formed according to the equation below. Throughout this thesis, the term reactive oxygen species (ROS) is used for oxygen-centered radicals.



Modified from[101].

During normal cell metabolism, superoxide anion radicals are generated during mitochondrial electron transport reactions reaching 1-2% of the total oxygen consumption[30]. In addition, superoxide may be produced by the metabolism of arachidonic acid and activation of xanthine oxidase[44;263] and by activation of NADPH oxidase in phagocytic cells. Superoxide does not cross intact cell membranes[96] but may penetrate to the extracellular space through anion channels[182]. Superoxide does not react significantly with DNA, proteins or phospholipids[136] but rapidly reacts with nitric oxide (NO) to cause the formation of peroxynitrite ($ONOO^-$). Peroxynitrite is increasingly recognized as a mediator of neurodegenerative disorders[82;292;315] and may damage and kill cells by inducing lipid peroxidation and protein tyrosine nitration[16;18].

Hydrogen peroxide easily penetrates lipid bilayers, acts like an oxidizing agent and is a relatively stable molecule, although not *per definition* a radical. H_2O_2 modulates kinases and phosphatase enzymes, but may not be toxic to living cells except in high and unphysiological concentrations[65;336]. Its probable role in the pathophysiology of CNS disorders is to act as a precursor of hydroxyl radicals.

Hydroxyl radicals ($OH^{\bullet}$) are extremely reactive, can react with practically any molecule present in cells and has a short half-life in biological tissue[301]. It is claimed to cause most of the pathology induced by ROS. No known enzymes produce $OH^{\bullet}$ by three-electron transfer but $OH^{\bullet}$ are formed when superoxide anion and hydroperoxide meet in solution according to the (Haber and Weiss[124]) reaction:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow OH^{\bullet} + OH^- + O_2$$

This reaction is extremely slow unless catalyzed by a transitional metal such as Fe^{2+} or copper (Cu^{2+}) ions (Fenton reaction).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^\bullet$$

Nitric oxide (NO), rather referred to as a reactive nitrogen species (RNS), has important physiological functions in the brain, such as the maintenance of BBB and regulation of resting CBF[269;318]. NO is synthesized from L-Arginine by at least three isoforms of nitric oxide synthases (NOS); neuronal NOS (nNOS; type I), inducible NOS (iNOS; type II) and endothelial NOS (eNOS; type III). Evidence suggests that eNOS activity is neuroprotective after acute brain injury, whereas iNOS and nNOS activity may be detrimental[158]. The expression of the isoforms of NOS are markedly altered after TBI, where the most marked changes occurred in the expression of iNOS in inflammatory cells invading brain tissue[100].

The brain is the tissue most vulnerable to oxidative damage because of its high rate of oxidative metabolic activity, intense production of reactive oxygen metabolites, relatively low antioxidant activity, low repair mechanism activity, nonreplicating nature of neuronal cells and the high membrane surface to cytoplasm ratio (reviewed by Shohami.[299]). In addition, the human brain contains a large amount of poly-unsaturated fatty acids, prone to lipid peroxidation reactions and, at least in some regions, high levels of iron and copper[160].

Examples of subcellular sites for ROS production are the electron transport chain in mitochondria, endoplasmatic reticulum and phospholipid bilayers[149;333]. ROS are formed at the endothelial level through the activation of xanthine oxidase and activation of plasma membrane NADPH oxidase in neutrophils[263]. Oxidative stress may further cause morphological and functional alterations in the mitochondria including damage to mitochondrial DNA[283;323]. In addition, ROS may cause changes in genes and gene products[91;232] and alter protein structure[41;94]. ROS are also prone to react with fatty acids and aromatic rings in biological systems[125], causing membrane phospholipid degradation[12;198]. An attack by a hydroxyl radical to a double bond in a fatty acid molecule yields a lipid hydroperoxide and a new carbon-centered radical.

Once the process has started by $OH^{\bullet}$ attack, iron-catalyzed chain reactions can be propagated without the participation of $OH^{\bullet}$, a chain reaction referred to as lipid peroxidation. Lipid peroxidation may proceed and cause severe membrane disturbance such as decreased fluidity, inactivation of membrane-bound receptors and enzymes and increased permeability to ions[123] until the chain reaction is broken by e.g., vitamin E.

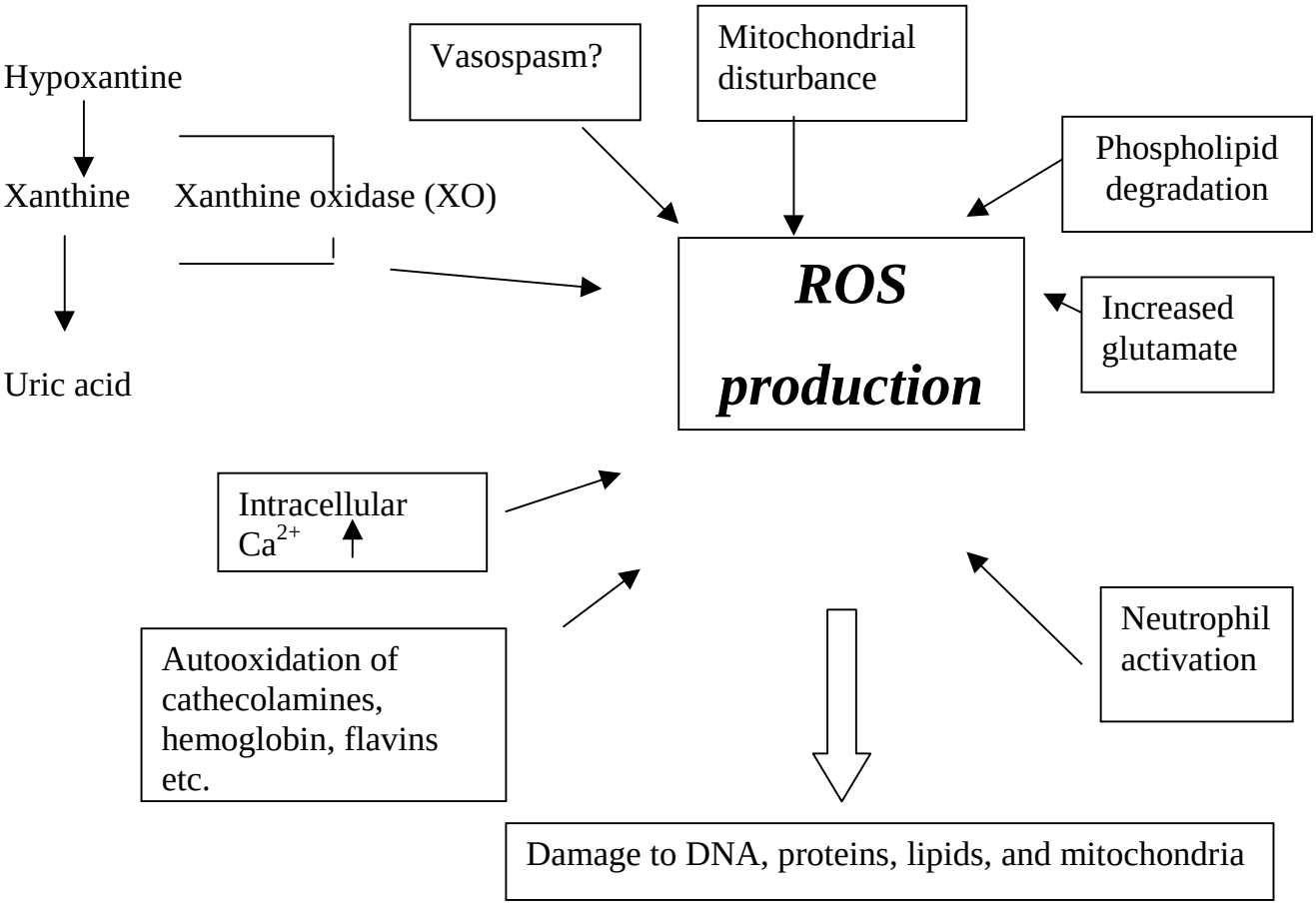


Fig. 1. A summary of mechanisms for ROS formation and the resulting damage to cellular components.

ROS defense systems

Oxidative stress is a situation when the production of ROS or any radical increases beyond the scavenging capabilities of the endogenous antioxidative protective systems. ROS damage may also be caused by inactivation of detoxification systems, consumption of antioxidants and failure to adequately replenish antioxidant in

damaged tissue[46]. Endothelial cells, neurons and glial cells all have intrinsic defense mechanisms against radicals. Three isoforms of superoxide dismutase (SOD) exists: mitochondrial Mn-SOD, cytosolic Cu/Zn-SOD and extracellular Cu/Zn-SOD[216] with manganese or copper and zinc ions at the active site. Through activation of SODs, $O_2^{\bullet-}$ is converted to H_2O_2. The H_2O_2 (and other lipid peroxides) thus formed is neutralized by glutathione peroxidase and catalase[94]. Catalase catalyzes the composition of hydrogen peroxide to water and oxygen and resides mainly in the peroxisomes though its activity in brain is low[273]. Glutathione (GSH) peroxidase resides in the mitochondrial matrix and the cytosol; it is mainly concentrated in astrocytes[299] and catalyzes the reaction

$$2\ GSH + H_2O_2 \longrightarrow GSSG + 2\ H_2O.$$

The enzyme glutathione reductase replenishes the oxidized form of glutathione (GSSG) back to GSH according to the reaction:

$$GSSG + NADPH, H^+ \longrightarrow 2\ GSH + NADP^+$$

Low molecular weight antioxidants (LMWA) such as α-tocopherol (vitamin E), ascorbate (vitamin C), uric acid, melatonin and histidine-related compounds are important antioxidants[285;299]. As an example, uric acid possesses about 60% of the total antioxidant capacity in plasma and has been estimated to scavenge 10-15 % of all hydroxyl radicals produced daily[5;21]. In addition, hemoglobin, albumin, transferrin and ceruloplasmin bind iron and copper preventing radical formation through the Fenton reaction[41]. Vitamin E is derived from the diet and does not readily pass the BBB but integrates well within the membrane phospholipid bilayer. Vitamin E is one of the most important radical-scavenging antioxidant within membranes[39] and effectively inhibits lipid peroxidation. When acting as a chain-breaker, vitamin E is converted to a radical that is regenerated to vitamin E by vitamin C. Ascorbate, vitamin C, together with glutathione, is the main antioxidant in the aqueous phase and also acts as a reducing agent[132;263]. Astrocytes are important in protecting neurons from ROS-induced damage in that they e.g., synthesize glutathione and recycles vitamin C[317].

Reactive oxygen species and TBI

ROS are thought to contribute to the secondary injury cascade after TBI. After TBI, there are several potential sites for ROS production, including mitochondrial leakage, increased free iron resulting from breakdown of extravasated hemoglobin, oxidation of catecholamines, breakdown of membrane phospholipids and infiltrating neutrophils[135;215;222]. The importance of mitochondrial ROS production in acute brain injury has recently received increasing attention[89]. Thus, *in vivo* conditions prevailing post ischemia or trauma favor a partial inhibition of the electron transport chain, producing a burst of ROS (i.e. $O_2^{\bullet-}$) in complex 1. ROS overproduction may contribute to post-traumatic mitochondrial dysfunction[149], implying that the mitochondria may be both an important source of ROS and a target for ROS-mediated damage. ROS may cause translocation of cytochrome (cyt) c from the mitochondria[89] and when the cyt c is lost, more ROS are produced in the mitochondria[243].

Moreover, increases in intracellular calcium[246;248] may induce ROS formation by activation of phospholipases, xanthine oxidase and nitric oxide synthase[212;226]. Increases in glutamate may generate ROS[291] and glutamate-induced Ca^{2+} toxicity was reduced if free radical generation was inhibited[74;255]. ROS may, in turn, cause further release of glutamate[258] and may adversely affect glutamate uptake[309]. These findings imply a link between glutamate- and ROS mediated damage after TBI.

Production of ROS has been reported to peak early after TBI[83;128;296]. Several ROS scavenging drugs or lipid peroxidation inhibitors have been evaluated in the treatment of experimental brain injury using e.g., edema[171;262;284], contusion volume[330], blood flow[66;241], motor deficits[129;139], survival[131;228], behavioral disturbances[53;139;171;207] or neurological status[139;284] as endpoints. These studies have included evaluation of lipid peroxidation inhibitors such as vitamin E and tirilazad, iron chelating agents such as desferoxamine (Desferal®), modified superoxide dismutase for improved BBB penetration[139], as well as several other drugs with antioxidative and radical scavenging properties[200].

So far, two compounds aimed at reducing radical damage after TBI have reached the clinical trial stage. PEG (polyethylene glycol-conjugated)-SOD reduced dead and

vegetative outcome in a phase II trial[242]. In the multicenter, phase III trial with 463 patients enrolled, no overall benefit was seen though there was a 9% reduction in mortality in PEG-SOD treated patients[348]. The lipid peroxidation inhibitor tirilazad mesylate was found neuroprotective in numerous studies of experimental brain injury and was evaluated in a European and a North American trial, in total including 1120 patients. No significant benefit of tirilazad treatment was observed using recovery or death as endpoints[218]. Because of lack of clinically available detection methods for ROS, the precise role for ROS in the clinical situation is unclear.

Detection of ROS

On account of the high reactivity and short half-life of ROS, indirect methods have been developed for their *in vivo* detection[92]. Some techniques detect signs of radical damage or stable compounds (adducts) formed when a parent compound reacts with radicals. These methods include morphological markers, protein and DNA oxidation products, determination of the activity of certain enzymes and measures to estimate ROS and NO production. A few studies have evaluated ROS formation after TBI[181]. Importantly, no *in vivo* method exists for routine use in clinical TBI. A survey of some methods used for ROS detection is summarized in Table 1. Although frequently encountering technical problems when applied to biological systems, electron spin resonance (ESR) and aromatic hydroxylation are probably the most specific methods for ROS detection[137]. Several aromatic compounds have been used to trap hydroxyl radicals in brain including salicylate (2-HBA), 4-hydroxybenzoic acid (4-HBA) and phenylalanine[97;313]. The salicylate (2-HBA) trapping technique is the most widely used for ROS detection *in vivo* and has previously been employed in experimental TBI together with microdialysis or after systemic administration[4;72;110;127]. Two adducts, 2,3- and 2,5-dihydroxybenzoate (DHBA), are formed upon reaction of salicylate with hydroxyl radical or peroxynitrite, However, concerns have been raised with regard to the specificity of the salicylate trapping technique in that enzymatic formation of 2,5-DHBA occurs at extracerebral sites by the cytochrome p450 system in the liver after systemic administration. 2,3-DHBA is also formed in the rat intestine, making it an unspecific marker[137;138;237]. Furthermore, the salicylate trapping method may have

Table 1. Survey of some techniques employed for ROS detection.

TECHNIQUE	SUBSTANCE MEASURED	MODEL	COMMENTS	REFERENCES
Electron Spin Resonance	Hydroxyl radicals	Weight drop or closed head injury, rat	Artifact prone in biological systems	Sen et al. 1994[296], Nisho et al. 1997[249]
Salicylate hydroxylation + MD	Hydroxyl radical (+ peroxynitrite)	Rats and mice, weigh drop and fluid percussion	2 adducts are formed	Hall et al. 1994[128] Globus et al. 1995[110]
4-hydroxy benzoic acid	Hydroxyl radical (+ peroxynitrite)	Focal ischemia, TBI rat	Systemic administration + MD	Gido et al. 2000[108] Fink et al. 1999[88]
Phenylalanine hydroxylation	Hydroxyl radical (+ peroxynitrite)	Myocardial ischemia , dogs	3 adducts are formed, not evaluated in TBI	Sun et al, 1993[313]
Decarboxylation of 14c ketoglutarate	H_2O_2 in microdialysate.	Global forebrain ischemia, rat .	Not evaluated in TBI.	Hyslop et al. 1995[157]
Cytochrome c electrode technique	Superoxide	Fluid percussion; rat	Increases for up to 3 hours post-TBI	Fabian et al. 1998[83]
Enzyme inactivation by ROS	Activity of glutamine synthetase	TBI, rats Tissue extracts	Decrease 3-18 d post-injury;	Jorgensen et al. 1997[164]
Activity of glutathione and catalase	Antioxidant activity in cortical tissue	TBI, rat	Tissue extracts necessary, increase post-injury	Goss et al. 1997[115]
Nitroblue tetrazolium (NBT); cranial window	Superoxide anions	a) NMDA vasodilation b) Cold injury, cats		a) Kulkarni et al. 2000[191] b) Ikeda et al. 1989[159]
Isoprostanes, i.e. prostaglandin F-2α	Lipid peroxidation	Trauma, rat	Increased 6 and 24 hours after TBI	Tyurin et al. 2000[324]
Fluorometric assay	Endproducts of NO, nitrite and nitrate	Cortical contusion injury	Increased up to 6 hours after TBI	Rao et al. 1998[272]
4-hydroxynonenal + immunoreactivity	Lipid peroxidation	Fluid percussion, rat	Increased up to 48 hours after FPI	Zhang et al. 1999[350]
Vitamin E consumption	Lipid peroxidation	Transient ischemia, rat	Reduced after ischemia	Yoshida et al. 1982[346]
Malondialdehyde (MDA) + TBAR	Lipid peroxidation	Various including TBI	Low specificity and artifact prone	Vagnozzi et al. 1999[326]
Microdialysis	H_2O_2	Spinal cord injury, rats	Increased 11 h post-injury	Liu et al 1999[203]
NOS immuno-histochemistry	Expression of the three isoforms of NOS	Weight drop injury, rat	Marked changes post-injury	Gahm et al. 2000[100]
Hydroethidine	Superoxide	Hippocampus, rat	Tissue slices	Bindokas et al.1996[26]
Dihydrorrhodamine	Peroxynitrite	*In vitro*	Oxidation to fluorescent rhodamin	Kooy et al. 1994[183]
Nitrotyrosine Immunohisto-chemistry	Peroxynitrite	Cortical contusion, mice	Increased in contused brain at 24 h	Whalen et al. 1999[335]
a) MtDNA-mutations b) 8-hydroxyguanine	Oxidative DNA damage	a) human head injury and b) transient ischemia, mice	Evidence for DNA damage	a)McDonald et al. 1999[224] b) Liu et al. 1996[204]
Protein oxidation products	Protein carbonyl content	Aging gerbil and mice	Decreased after PBN administration	Dubey et al. 1995[73]
Uric acid oxidation products; HPLC	a) Parabanic acid b)Allantoin	Human acute brain injury; microdialysis	Unstable at physiological pH.	a) Hillered and Persson,1995[150] b)Marklund et al. 2000[214]

several negative side effects when considering its clinical utility as salicylate may affect vascular tone, platelet function and inflammatory responses through its effect on the cyclo-oxygenase pathway[6;43;63].

Another aromatic compound, phenylalanine gives rise to three isomeric tyrosines upon reactions with $OH^{\bullet}$ and has not been evaluated in TBI.

A third aromatic compound, 4-hydroxy benzoic acid (4-HBA) forms only one stable adduct (3,4-DHBA) upon reaction with ROS. 4-HBA is found endogenously and no adverse side effects are known. 4-HBA, together with microdialysis as a tool for ROS detection after TBI, is evaluated in the present thesis.

Nitrones and acute brain injury

The spin-trapping agent α-phenyl- *tert* -N-butyl nitrone (PBN) was originally used for the detection of free radicals *in vivo* and *in vitro* coupled with ESR. It was accidentally found that its ability to form stable adducts with ROS might be neuroprotective[252]. Since then, PBN has been evaluated in numerous studies of acute brain injury and neurodegenerative disorders (e.g.,[42;257;351]). Nitrones are well-known spin-trapping agents and have been shown to reduce brain hydroxyl radical formation after TBI[296] and following glutamate or malonate exposure in the rat striatum[86;293].

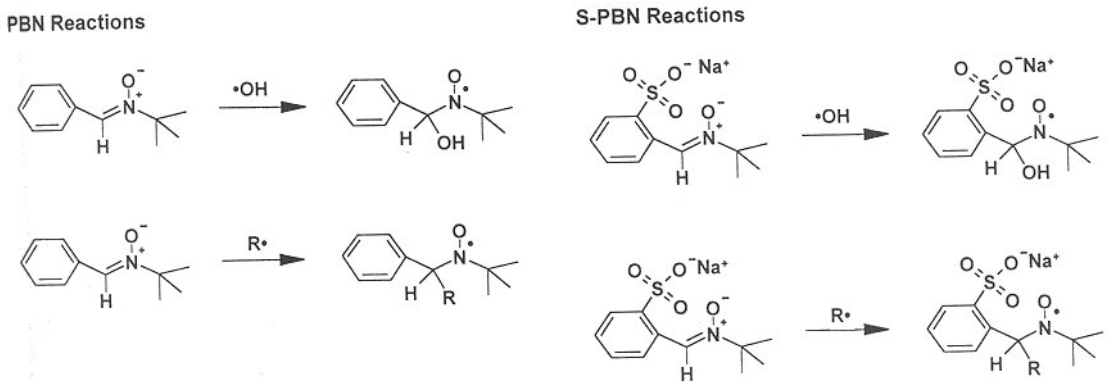


Fig. 2. The chemical structures of PBN and S-PBN and the radical-nitrone reaction products.

When compared with PBN in models of focal ischemia, sulfonated derivatives of PBN, sodium 2-sulfophenyl-N-*tert*-butyl nitrone (S-PBN) and disodium 2,4-disulfophenyl-N-*tert*-butyl nitrone (NXY-059) were found to have similar or even superior neuroprotective properties. For example, S-PBN and PBN equally attenuated infarct volume following focal ischemia[344]. Additionally, at an equimolar dose of PBN, the polar NXY-059 was more efficacious than PBN with a similar time-window (up to 3 h but not 6 h) after focal ischemia despite a very low brain penetration of NXY-059[193]. NXY-059 is presently in a phase II clinical trial for stroke. Because of the potent effects of nitrones with low BBB penetration, the blood-endothelial interface was considered important in the pathobiology of focal brain ischemia. Neither PBN nor S-PBN has been evaluated in TBI using clinically relevant endpoints such as morphological and functional outcome measures. In the present thesis, the aim is to use PBN and S-PBN as tools for studying the importance of ROS in the injury process after TBI.

EXPERIMENTAL MODELS OF TBI

Experimental models of TBI have been developed to permit measurement of the pathophysiological and neurobehavioral responses to trauma[239]. Because of the complexity of human TBI, no existing experimental model mimics all features of human head injury. Hence, there are numerous experimental models in existence today[312]. Common models employed in neurotrauma include head acceleration/deceleration models, models causing rotation of the head and cortical impact devices[104;312]. These models have been developed to mimic diffuse axonal damage, cortical contusions or a combination type of injury. There are many morphological and physiological similarities between the clinical and experimental setting, although important differences exist, including the lack of TBI models producing long-lasting coma.

Two decades ago the controlled cortical contusion injury (originally described by Feeney[85]) was introduced and gained wide popularity in several laboratories, including our own. In this model, a weight is dropped onto a piston resting on the exposed cortex

where the severity level may be varied as needed by changing the depth of indentation of the cortex. This model typically produces a focal contusion with hemorrhages, apoptosis and necrosis[302], causing the formation of a cortical cavity at higher injury levels (2.5 mm[248]). In the mild and moderate (1.5 and 2.0 mm) trauma, no cavity is seen and only mild morphological damage ensues. As an example, in the 1.5 mm compression level, a few scattered eosinophilic neurons are noted at three days after injury in the center of the impact[246], but at the perimeter of the impact site there are spongiosis, distorted neurons, leukocyte infiltration, loss of neurons and a disturbance of the BBB. Neurological deficits are mild, consisting of only impairments of contralateral limb fore-limb flexion and hind limb retraction, all of which subside within a few days[247].

The second TBI model used in the present thesis, the lateral (parasagittal) fluid percussion injury model was introduced in 1989[229]. This model has gained acceptance world-wide since its introduction. With this model, a craniotomy is made and a pressure wave with a duration of 21-23 ms is transmitted to the brain of the rat producing a focal contusion and, additionally, a displacement of brain tissue that produces a more diffuse injury, including bilateral hippocampal damage. Fluid percussion injury causes neuromotor deficits[229], alterations in hippocampal seizure threshold[208], changes in ICP and EEG activity[229], regional edema[308] and cognitive deficits[140;264;305]. The frequency of injured neurons is high in the ipsilateral cortex, hippocampus, hilus of the dentate gyrus and the thalamus although some damage is seen in the contralateral hippocampus and cortex as well[146]. The cortex, CA3 subfield[58;147], and hilus of the dentate gyrus are regarded as the most vulnerable regions in this model[226]. Furthermore, a wide-spread BBB disturbance is seen as demonstrated by the leakage of horseradish peroxidase[288].

AIMS

The general aim of this work is to evaluate the role of reactive oxygen species (ROS) in traumatic brain injury. The nitrones α-phenyl-N-*tert* butyl nitrone **(**PBN) and 2-sulfo-phenyl-N-*tert*-butyl nitrone (S-PBN) were used as tools to study the influence of ROS on the brain injury process and outcome after TBI in rats.

The specific aims were as follows:

-to investigate if a single dose of PBN administered i.v. 30 min before a controlled cortical contusion injury influences morphological and cognitive outcome;

-to investigate if PBN and S-PBN treatment started 30 min after fluid percussion injury followed by an i.v. infusion for 24 h influence morphological and functional outcome;

-to investigate if the neuroprotective effects seen with PBN pre-treatment were due to an influence on the number of apoptotic neurons after a moderate controlled cortical contusion injury;

-to investigate if PBN and S-PBN influence changes on regional cerebral blood flow and glucose uptake in cortex and hippocampus induced by fluid percussion injury;

-to evaluate 4-hydroxybenzoic acid in conjunction with microdialysis as a method for ROS detection after controlled cortical contusion injury; and

-to investigate if PBN and S-PBN influence ROS formation after controlled cortical contusion injury and to analyze plasma and brain concentrations of PBN and S-PBN after injury.

MATERIAL AND METHODS

SURGICAL PROCEDURE AND DRUG ADMINISTRATION

All procedures were performed according to protocols approved by the local ethics committee of Uppsala University. Every effort was made to minimize animal suffering and to reduce the number of animals used in each experiment. A detailed description of the experimental methods is found in each paper.

Male Sprague-Dawley rats were induced in Halothane in oxygen, tracheally intubated and mechanically ventilated with a mixture of inhaled O_2/N_2O (30/70%) and isoflurane (1,2-1,4 %). In Paper IV, a single dose of sodium pentobarbital (60 mg/kg) intraperitoneally (i.p.) was used. Full physiological monitoring was used including monitoring of PO_2, PCO_2 and pH and (in Paper II and IV) blood glucose. The length of apnea and time to return of the toe-pinch reflex was measured.

For production of FPI injury (Papers II and IV), a lateral 4.8 mm diameter craniotomy was made at bregma -2.0 mm to – 7.0 mm, a trauma cap filled with saline was placed over the craniotomy that was held in place by tissue adhesive and cement. The animals were disconnected from the ventilator and taken to the trauma device, and a 2.20-2.60 atm injury was delivered. The length of apnea and loss of the toe-pinch reflex was measured. After resumption of breathing, the animals were re-connected to the ventilator and anesthesia continued.

For production of controlled cortical contusion injury (Papers I, III, V and VI), a craniotomy (6×9 mm) centered over the right parietal cortex at bregma –3.5 mm and 3.5 mm lateral to the midline was made. A 21 g weight was dropped from a height of 35 cm through a guiding tube onto a 4.5 mm diameter piston resting on the exposed dura. The pistons were manufactured to allow a maximum compression of 1.5 mm (mild injury), 2.0 mm (moderate injury) or 2.5 mm (severe injury).

PBN and S-PBN were dissolved in isotonic saline the day of the experiment (yielding solutions of 15 mg/ml and 23.5 mg/ml, respectively). Equimolar doses of PBN (30 mg/kg) or S-PBN (47 mg/kg) were slowly injected i.v. 30 minutes prior to injury (Paper I, III-IV and VI). In Paper II, an equimolar bolus dose of PBN or S-PBN (30

mg/kg or 47 mg/kg, respectively) was given 30 min post-injury followed by an i.v. infusion of 30 mg/kg/hr or 47 mg/kg/hr, respectively, for 24 h.

MORPHOLOGICAL AND FUNCTIONAL OUTCOME (PAPERS I-III):

The formation of declarative memory depends on a system of anatomically related structures in the medial temporal lobe, including the hippocampal region and the adjacent entorhinal, perirhinal and parahippocampal cortices[353]. Hippocampal damage is frequently observed in TBI and correlates with the severity of post-traumatic memory dysfunction, persisting up to one year after injury[147;264]. CA3, because of its developed intrinsic connections, may be particularly important in memory retrieval[322] and is sensitive to damage after TBI. Because of the frequent occurrence of cognitive dysfunction and hippocampal pathology after human TBI, there is a strong need for assessment of cognitive function in the experimental setting. The Morris Water Maze (MWM) was introduced in the 1980´s and has become a widely used instrument for the assessment of cognitive dysfunction across many species and neurodegenerative disorders[32;238]. Our maze system consists of a dark pool with a diameter of 1.4 m filled with water and a 12 cm diameter platform submerged 2 cm below the surface. The rats were trained with 4 trials per day for 5 consecutive days (days 11-15 post-injury)[139]. Various variables on every trial (swim speed, latency, path length, pattern, etc.) were measured using a computer-linked video system with software allowing detailed analysis of every trial.

At days 1, 4 and 8 post-injury, a modified neurological scoring system[19] was used for assessment of motor function (Paper II).

The histological evaluation was based on the loss of microtubule-associated protein-2 (MAP-2) immunoreactivity (Paper I) or hematoxylin and eosin (H/E) staining (Paper II and III). MAP-2 is one of a family of MAPs maintaining the structural integrity of the neuronal cytoskeleton. MAP-2 immunohistochemistry has been used to quantify lesion volume in TBI[148] and correlates well with H/E staining[61]. H/E, used in Paper II and III, is a widely used tool for the assessment of routine morphology.

For MAP-2 immunohistochemistry, paraplast embedded brains were sectioned into serial 6 µm coronal sections at bregma –1 to –6 mm. MAP-2 immunoreactivity was performed according to Lewén et al[199]. In brief, sections were incubated with a monoclonal antibody against MAP-2, exposed to the secondary antibody and the reaction product visualized with diaminobenzidine.

In Paper II, brains were rapidly frozen in isopenthane at -60 °C. Fourteen µm serial coronal frozen sections were made and stained with hematoxylin and eosin (H/E) for routine morphology. For determination of ipsi- and contralateral hemispheric volume, the hemispheric area was determined with a computerized image analysis system and multiplied by the distance between the sections (Papers I -III).

DETECTION OF APOPTOSIS (PAPER III)

For detection of apoptotic cells, the TUNEL technique for detection of DNA strand breaks and strict light microscopic criteria was used at bregma – 3.5 mm. Double staining with the nuclear dye Hoechst 33258 and immunohistochemistry for the active form of caspase-3 was performed. To identify the cellular nature of apoptotic cells, double staining with the neuronal marker anti-Neu-N antibody was performed and the sections were photographed using the fluorescence microscope. In each animal (12-72 h post-injury), all TUNEL positive cells were counted in four consecutive coronal sections by a blinded observer.

ESTIMATION OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) AND GLUCOSE UPTAKE (PAPER IV)

In this study, an approach of relative quantitation for estimation of glucose uptake as measured by [^{18}F] Fluoro-2-deoxyglucose (FDG) autoradiography was taken at 42 min or 12 h after the injury. FDG is a widely used tracer for estimation of the cerebral metabolic rate of glucose (CMR_{glc}). FDG (15-20 MBq/ml) was injected i.v. and the animals were decapitated 45 min after the injection of the tracer.

For assessment of rCBF, 99mTc-hexamethylpropylene amine oxime [^{99m}Tc]HMPAO was used. [^{99m}Tc] HMPAO is polar and distributes in brain in proportion to CBF[209], crosses the BBB to reach the intracellular compartment and decomposes to a

hydrophilic compound that is trapped intracellularly. It provides a simple and reliable way of estimating CBF by means of autoradiography and is widely used in clinical TBI[1;343]. Examples of early changes in FDG and HMPAO uptake are shown in Fig 2.

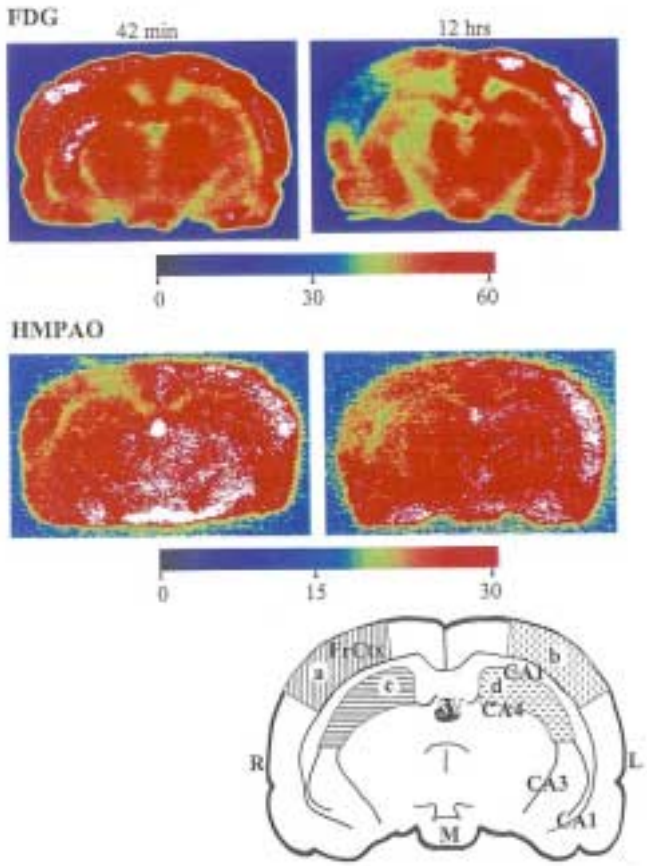


Fig.3. Autoradiographic images of FDG and HMPAO at 42 min (left) and 12 h (right after fluid percussion injury). The images were taken at 5 mm posterior to bregma. CA1-4; fields 1-4 of Ammon's horn. R- right, L- left, 3V, third ventricle, M, corpora mammillaria. FrCTX, frontoparietal cortex; trauma site (a) and (b) for the opposite cortical ROI. (c) and (d) denotes the ipsi- and contralateral hippocampal ROIs, respectively.

[^{99m}Tc] HMPAO was injected intravenously and the rats were decapitated 5 min after the injection of tracer. After decapitation, coronal frozen sections were made from bregma 2.0 mm to –8.0 mm. The sections were exposed for 4 hours (FDG) or 12 hours ([^{99m}Tc] HMPAO) to phosphor imaging plates. The imaging plates were scanned with a laser beam and quantified by normalizing the uptake pixel-value in regions of interest (ROI) to the administered volume of the tracer and body weight of the animal.

MICRODIALYSIS PROCEDURE

The extracellular fluid (ECF), considered to compose 17-20% of the volume of the brain, is a site for important pathophysiological events in TBI[259]. Intracerebral microdialysis is a technique by which important chemical changes in the ECF can be measured over time. Since the mid-1980´s there has been a substantial increase in the number of publications using microdialysis; application to the acutely injured human brain was made a decade ago[259]. The MD probe consists of a semipermeable membrane allowing free diffusion of substances between the perfusion solution and the extracellular fluid of the tissue surrounding the probe. Low-molecular substances (molecular cut-off usually 20 kDa) can then be collected and analyzed from the fluid (dialysate) leaving the probe.

3 mM 4-hydroxybenzoic acid (4-HBA) dissolved in aCSF in the perfusate was used in Papers V and VI. When 4-HBA reacts with ROS, one stable adduct, 3,4-DHBA, is formed, retrieved from the dialysate and analyzed by HPLC (see below). Thirty min samples were collected for 2 h before injury and for 3 h after trauma. For comparison, the salicylate trapping method (3 mM salicylate in the perfusate) was used in sham or severely injured rats.

HPLC ANALYSIS

Because of its sensitivity and its wide application to biological systems, high performance liquid chromatography (HPLC) is unquestionably the most widely used of all separation techniques. For a detailed description of our HPLC system, see Papers V and VI. The HPLC system was equipped with a metal free injector and all connections were metal free in order to prevent autooxidation of 4-HBA.

Microdialysis samples were analyzed for 3,4-DHBA or 2,3- and 2,5-DHBA by a reversed phase HPLC system coupled with electrochemical detection.
Brain tissue was homogenized, centrifuged and filtered and analysis of 4-HBA and salicylate in brain tissue samples was done using an HPLC system with UV detection. PBN and S-PBN in plasma and brain tissue were analyzed by HPLC and UV detection utilizing the same instrumentation as used for 4-HBA. The minimum detectable concentration of PBN in plasma was 0.35 μg/ml and of S-PBN 0.62 μg/ml. The limit of detection in brain was 2.0 μg/g for PBN and 0.3 μg/g for S-PBN.

STATISTICAL METHODS

Two-way analysis of variance (ANOVA) was used for group comparisons followed by a post-hoc test (Bonferroni or Fisher's PLSD) if statistically significant at $p<0.05$. For the neurological data in Paper II, non-parametric methods were used. A statistical program (StatView 4.51, Abacus Concepts Inc, Berkeley, CA) was used for all handling and statistical analysis. All data are presented as Means ± Standard Deviations (SD) or Standard Errors of the Mean (SEM). A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

RESULTS OF NITRONE TREATMENT ON MORPHOLOGICAL AND FUNCTIONAL OUTCOME AFTER TBI (PAPERS I AND II)

PBN pre-treatment: controlled cortical contusion injury

To study the influence of ROS on cognitive and morphological outcome after a mild and moderate controlled cortical contusion injury, PBN pre-treatment (30 mg/kg i.v. 30 min prior to injury) was used. Endpoints were lesion volume and loss of hemispheric tissue at day 15 and MWM performance at day 11-15 post-injury.
No cortical cavity or diffuse atrophy of the hemisphere ipsilateral to the trauma site was noted in mildly traumatized animals. In contrast, the severe injury (2.5 mm

depression) caused a large cortical cavitation, and a marked ipsilateral hemispheric atrophy. PBN pre-treatment attenuated the morphological disturbances seen in the severely injured groups but no change was observed after PBN treatment in the mildly traumatized groups.

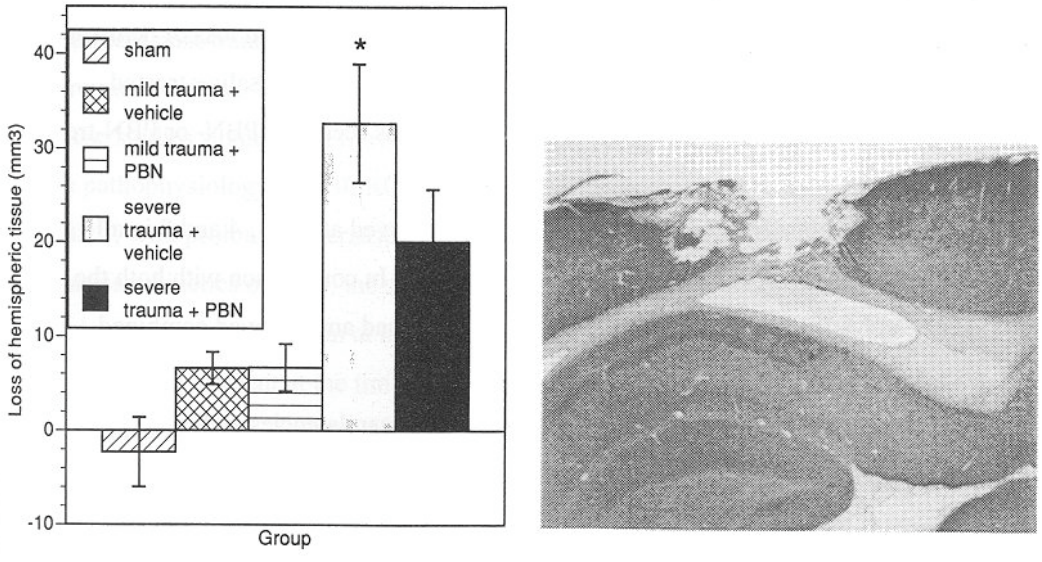


Fig.4. Loss of hemispheric tissue compared between the treatment groups: To the right is an example of a severe (2.5 mm depression) injury.

When assessing total mean latency and path length, performance in the MWM was significantly impaired in the severely traumatized groups as compared with the mildly traumatized and sham animals. In the severely traumatized groups, PBN pre-treatment significantly improved MWM performance (total mean latency and path length) as compared with saline-treated animals. No difference was found in swim speed between groups, implying that the observed changes were not due to neurological impairment.

This Paper demonstrates that PBN pre-treatment attenuates morphological and cognitive damage after TBI, implying that ROS are involved in the pathophysiology of TBI.

PBN and S-PBN post-treatment, fluid percussion injury

In this study, PBN and S-PBN (30 mg/kg or 47 mg/kg, respectively) are administered i.v. at 30 min) after a fluid percussion injury, followed by a 24 h infusion (30 mg/kg/hr or 47 mg/kg/hr). Endpoints were morphological outcome (day 15), neurological evaluation (day 1,4 and 8) and MWM performance (day 11-15) post-injury.

Both drug-treated groups equally showed a significant reduction in loss of hemispheric tissue and improved the hemispheric area ratio as compared with saline-treated animals. No significant reduction in lesion volume was seen in S-PBN- or PBN-treated animals.

A significant impairment in motor function was observed at day 1, 4 and 8 in all traumatized groups compared with the sham animals. In comparison with both the saline- and S-PBN-treated rats, the PBN-treated rats had an improved combined neurological score.

FPI induced a cognitive deficit with increased total mean latencies and increased latencies on day 11, 12 and 15 post-injury as compared with sham injured controls.

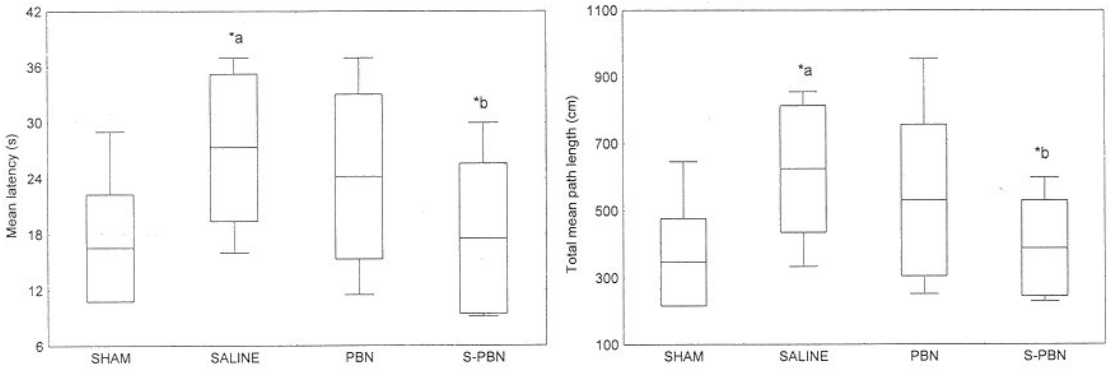


Fig.5. Morris Water Maze performance as measured by the mean latency (left) and mean path length (right) to reach the hidden platform.

The S-PBN-treated rats had a shorter mean total latency and path length in comparison with the other trauma groups and performed equal to sham-operated controls. No improvement in PBN treated animals was found. There was no difference in mean swim speed between the treatment groups. In conclusion, this Paper shows that nitrone treatment post-trauma attenuates morphological and functional outcome after fluid percussion injury.

These studies suggest that nitrone scavengers positively influence factors important in the pathophysiology of TBI. ROS formation has been shown to be an early event after TBI[126], with prolonged increases seen for several hours after TBI[83;110]. Because of the pharmacokinetics of PBN, the single pre-injury dose of PBN used in Paper I reaches peak concentration in brain at the time of injury[47;48]. Thus, a significant amount of PBN is likely present at the time of peak ROS formation as well as for the first few hours after TBI. The time point for drug delivery in Paper II was chosen to represent the earliest time point that a TBI victim would be given a neuroprotective drug at the scene of injury. Numerous studies of TBI using ROS scavengers exist, e.g., showing reduction of edema[262], attenuation of hypoperfusion[241] and reduction in BBB permeability[306] but only a few have reported an improvement in cognitive function. As an example, PEG-SOD (Pegogortein®), ultimately reaching clinical trials, improved motor function after FPI in rats; however, it did not improve MWM performance[139]. Nevertheless, scavenging of superoxide[171] or chelation of free iron[207] was shown to attenuate cognitive deficits after experimental TBI. In addition, *in vitro* work demonstrated that hydroxyl radicals preferentially cause CA3 damage and superoxide anions CA1 damage, effects that were attenuated by S-PBN[337]. The present result suggests that the process that causes widespread injury is delayed beyond the first 30 minutes after TBI. In view of the commonly seen cognitive disturbances in human TBI, drugs that positively influence cognitive and emotional disturbances are needed. The observed ability of S-PBN to attenuate cognitive disturbances raises the possibility that nitrones may have a potential for the clinical treatment of TBI.

Mechanisms of action of nitrones in the treatment of TBI (PAPERS I-IV, VI)

Although ROS have been implicated with normal cell-to-cell signaling and are formed for useful purposes[320], excess ROS production is generally considered harmful. In addition, the use of transgenic animals have consistently shown that overexpression of SODs is neuroprotective, and that reduced expression of SODs worsens outcome after focal ischemia[200;297;298]. The present results suggest that nitrones are neuroprotective after TBI in rats and may be compounds attractive for the clinical setting because of their low toxicity and large time window as shown in ischemia models. NXY-059 showed marked neuroprotection in permanent focal ischemia in a primate model starting 5 min after the onset of ischemia, protecting both gray and white matter and reducing the contralateral hemiparesis[217]. Nitrones, however, are not considered particularly effective as scavengers and alternate mechanisms of actions for nitrones (e.g., influence on inflammatory mediators[184;265], transmittor systems[116], or an influence on Ca^{2+}-channels[9]) have been suggested. Additionally, PBN was shown to condition the brain of old rats to resist ischemia even when PBN had not been administered for several days[90], suggesting other mechanisms than merely reduction of free radicals. Nitrones may influence several intracellular cascades as NXY-059 was shown to attenuate the decrease of the serine-threonine kinase Akt in ischemic tissue and enhance extracellular signal-regulated kinase (ERK) concentrations in microglial and endothelial cells after transient ischemia[213]. In addition, in a study of isolated macrophages, PBN suppressed cyclooxygenase (COX)-2 and iNOS mRNA levels[184]. Finally, PBN is known to attenuate secondary mitochondrial deterioration after focal ischemia and improve energy charge[93;192], likely acting on complex 1[145]. After a controlled cortical contusion injury, PBN attenuated the rise in extracellular lactate and glycerol, indicating an improved metabolic function and decreased phosholipid degradation[198]. These studies suggest a protection of mitochondria by nitrones in acute brain injury.

Tight junctions are present at the cerebral endothelial cells of brain capillaries that restrict the transport of hydrophilic drugs[62]. Endothelial cells have a high activity of drug metabolizing enzymes, such as the cyt p450[194], and there are data indicating that

TBI alters the BBB with changes in metabolism and clearance of drugs[28]. For example, PEG-SOD concentrations were 6-10 times higher in brain tissue of traumatized rats compared with controls[345]. The transport of drugs across the BBB may be restricted by e.g., P-glycoprotein, a transmembrane transport protein that is expressed on the luminal side of endothelial cells[107]. In Paper VI, we did not witness increased concentrations of nitrones in traumatized tissue, but our results imply that measurements of brain penetrations of neuroprotective drugs are important.
PBN has a half-life in plasma of approximately 3 h, S-PBN only 9 min, and no metabolites of PBN or S-PBN possessing neuroprotective properties are known. However, recent cell culture work[11] found that a degradation product of PBN, *N-t*-butyl hydroxylamine, appearing in old solutions stored for several months, was more potent than PBN in delaying senescence in cultured lung fibroblasts. The importance of degradation products of PBN *in vivo* is unknown but a contribution to the present results cannot be excluded. However, in all studies presented in this thesis, PBN or S-PBN solutions were made fresh on the day of the experiment in an attempt to keep the concentration of degradation products low.

PBN PRE-TREATMENT AND THE EFFECT OF THE NUMBER OF APOPTOTIC NEURONS (PAPER III)

In this Paper, the neuroprotective effects of PBN pre-treatment (30 mg/kg, administered i.v. 30 min before injury) were compared with the number of apoptotic cortical cells 24 h after a moderate controlled cortical contusion injury.
In saline-treated animals after moderate injury, several cells in the perimeter of the lesion were TUNEL positive at 12 to 72 h, peaking at 24 h. Double staining with Hoechst, a nuclear marker, revealed that the TUNEL staining resided in the nucleus, suggestive of apoptosis. Double staining against the neuronal marker, Neu-N, revealed that a major part of TUNEL positive cells consisted of neurons. A large number of neurons were positive for p17 and p12 segments of activated caspase-3 at 24 h suggesting ongoing biochemical apoptosis.
In PBN pre-treated rats, there was an 80 % increase in the number of TUNEL positive cells as compared with saline-treated animals. The number of caspase-3 positive cells

showed the same increase in PBN-treated as in saline-treated animals and Neu-N double staining indicated that these cells were neurons.

This study demonstrated a paradoxical increase of apoptotic neurons in PBN-treated animals and indicate that an increase in apoptosis may be compatible with an overall neuroprotective effect after TBI. These results differ from a contusion injury in immature rats, where a decreased number of apoptotic cells were seen after S-PBN treatment[266]. Differences in drug effect, the age of the animals, or both could explain these differences. Additionally, several morphological features and biochemical factors are similar in necrosis and apoptosis perhaps representing only the extreme ends of a broad range of possible morphological and biochemical deaths[195]. The pathway that is chosen may ultimately be contingent on injury severity, where mild injury causes more apoptosis, whereas in severe injury necrosis is the predominant pathway. In saline-treated animals at this injury severity level, necrosis may be the predominant mode of cell death, causing death of neighboring cells because of leakage of glutamate and lysozymal enzymes. Although no attempt was made to count necrotic cells, a decrease is likely due to the overall tissue sparing effect seen with PBN.

Oxidative damage as a result from ROS has been suggested to contribute to the induction of neuronal apoptosis through DNA damage[120;271]. ROS may be an important down-stream mediator of cell death as caspase-1 inhibition decreased ROS production and cell death[88]. Additionally, other pro-oxidants (e.g., diamide and etopside, NO donors and peroxynitrite) induce apoptosis[82;286;315]. Furthermore, overexpression of SOD 2 prevents neuronal apoptosis by reducing peroxynitrite formation, lipid peroxidation and mitochondrial dysfunction[172] and superoxide dismutase depletion causes activation of caspases and apoptotic cell death[254]. These studies indicate that ROS contributes to the induction and execution of the apoptotic pathway and would imply that PBN, in reducing ROS, would reduce apoptosis. Accordingly, PBN was found to ameliorate apoptosis after doxorubicin administration in cardiomyocytes[185]. However, inverse results were obtained in a model of pneumococcal meningitis in which PBN aggravated hippocampal apoptosis[206]. Our results show an increase in

apoptotic neurons after PBN administration in TBI at a dose that was shown to be neuroprotective. The plausible reasons for the paradoxical increase in the number of apoptotic neurons following PBN treatment are discussed in the next section.

Apoptosis- friend or foe in injured brain?

After FPI, caspase-1 and 3 mRNA are upregulated and activation of caspase 3 and 9 occurs[342]. Treatment with inhibitors of apoptosis such as z-DEVD-fmk in rats attenuated cortical injury although no improvement in motor skills was noted[51]. Additionally, induction of apoptosis by staurosporin increased lesion volume after focal ischemia[50]. These studies indicate that inhibiting apoptosis would be beneficial. However, mice deficient in the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) do not have a reduced number of apoptotic neurons[81] despite a reduced lesion volume after focal ischemia[78]. Several factors may indicate a shift from necrosis to apoptosis by PBN. Intracellular ATP levels may be a crucial event determining if necrosis or apoptosis ensues after an insult to a tissue where intracellular ATP levels less than 15 % cause necrosis in which higher ATP levels provide enough energy for the apoptotic pathway[76;202]. PBN may improve blood flow and energy production (*vide infra*), attenuating mitochondrial dysfunction and improve energy charge with increased intracellular ATP levels.

Finally, concentrations of H_2O_2[102] and calcium[35], and the degree of mitochondrial damage[190] may determine which pathway is chosen. An effect of PBN on any or all of these biochemical factors may result in a milder form of injury with an increase in apoptosis following TBI. For optimal results, a combination of agents that reduce both necrosis and apoptosis would be beneficial.

CHANGES IN CEREBRAL BLOOD FLOW, GLUCOSE METABOLISM AND THE EFFECT OF PBN AND S-PBN PRE-TREATMENT AFTER FLUID PERCUSSION INJURY (PAPER IV)

In this Paper, relative changes of hippocampal and cortical rCBF and glucose uptake was analyzed through HMPAO and FDG autoradiography, respectively, at 42 min and 12 h after fluid percussion injury. The involvement of ROS on the trauma-induced changes was assessed by administering PBN (30 mg/kg) or S-PBN (47 mg/kg) i.v. 30 min before injury.

Cortical changes: rCBF

The fluid percussion injury caused an early (42 min) mean decrease of 20% in ipsilateral rCBF followed by an even more marked reduction at 12 h as compared with sham animals. PBN and S-PBN treatment equally increased early ipsilateral rCBF to the level of sham-operated animals. PBN-treatment only attenuated the decrease in ipsilateral rCBF at 12 h. No early contralateral changes in rCBF were found but at 12 h there was a significant reduction of contralateral rCBF in S-PBN- and untreated traumatized rats as compared with sham animals.

Hippocampal changes: rCBF

TBI did not cause any significant reduction in early (42 min) ipsi- or contralateral hippocampal rCBF, but at 12 h there was a reduced ipsi- and contralateral rCBF in all trauma groups, with no significant changes after PBN and S-PBN treatment.

S-PBN-treated rats had increased early contralateral hippocampal rCBF as compared with the untreated traumatized group and sham animals.

Cortical changes: glucose uptake

The trauma significantly increased the early (42 min) ipsilateral glucose uptake that was markedly decreased at 12 h. No contralateral changes were seen at any time point. PBN attenuated the early ipsilateral increase to the levels of sham animals, being significantly lower than untreated traumatized rats. Additionally, although not

reaching statistical significance, the S-PBN-treated animals had an attenuated glucose uptake ($p=0.05$). On the contralateral side, S-PBN-treated rats, in comparison with sham animals and untreated traumatized rats, showed an early decrease in glucose uptake and an increase in glucose uptake at 12 h.

Hippocampal changes: glucose uptake

TBI caused an early increased ipsilateral glucose uptake as compared with sham animals ($p<0.05$), being significantly attenuated by S-PBN and PBN treatment. In contrast, at 12 h, traumatized animals, when compared with sham animals, had a marked decrease in late glucose uptake ($p<0.05$). At this time point, S-PBN pre-treatment significantly attenuated the decrease in glucose uptake as compared with untreated traumatized and PBN-treated animals ($p<0.05$).

	Ipsilateral glucose uptake, changes relative to controls	Ipsilateral rCBF, changes relative to controls
Cortex. 42 min post injury	Saline + 23 % *a	Saline – 19 %
	PBN + 2% *b	PBN + 23 % *b
	S-PBN + 10 %	S-PBN + 26 % *b
Hippocampus 42 min post-injury	Saline + 25 % *a	Saline – 6 %
	PBN + 4 % *b	PBN + 23 %
	S-PBN ± 0% *b	S-PBN + 28 %
Cortex 12 h post injury	Saline – 59 % *a	Saline – 61 % *a
	PBN – 46 % *a	PBN – 45 % *a,b
	S-PBN – 37 % *a	S-PBN – 53 % *a
Hippocampus 12 h post-injury	Saline – 34 % *a	Saline – 42 % *a
	PBN – 17 %	PBN – 33 % *a
	S-PBN + 14 % *b	S-PBN – 36 % *a

Table 2. Relative increase or decrease (in %) in traumatized animals compared with sham-operated controls. A significant difference between (a) sham-operated and (b) untreated traumatized animals is indicated by *.

In the contralateral hippocampus, no significant change in glucose uptake as compared with sham animals was seen at either time point. S-PBN treatment significantly

decreased early (42 min) and increased late contralateral glucose uptake as compared with sham and untreated traumatized rats.

These results indicate that marked changes in rCBF and glucose uptake ensue after FPI. S-PBN and PBN treatment attenuated and, occasionally, normalized these changes. The ability to attenuate decreases in rCBF and changes in glucose uptake may indicate an improved delivery of substrate for ATP production and less compromised glucose metabolism through an attenuation of mitochondrial dysfunction. Because of the observed neuroprotective properties seen with nitrone scavengers in TBI (present thesis) and ischemia, the changes observed here are likely beneficial for the injury process in TBI. As S-PBN has poor BBB penetration, these result imply that the blood-endothelial interface plays an important role in the TBI-induced changes in rCBF and glucose uptake.
It is not clear if hyperglycolysis *per se* mediates secondary tissue injury or if it merely represents an attempt for an energy-compromised tissue to increase ATP production. Hyperglycolysis combined with a decrease in oxidative metabolism causes lactate accumulation. Although lactate is used as a substrate for neurons[89], excess lactate is toxic because of a lowering of pH. In clinical studies, lactate has been shown to correlate with ICP elevations, neurological deterioration and poor outcome[113;275].
The early hyperglycolysis was followed by a decrease in rCBF and glucose uptake, suggestive of a depressed metabolic state which has been linked to reduced neuronal activity. In the hippocampus, there were bilateral reductions in rCBF and glucose metabolism bilaterally at 12 h, a finding consistent with the bilateral functional disturbance noted in the hippocampus after FPI. It is likely that the observed impairment in mitochondrial capacity post-TBI[340] contributes to the observed metabolic depression post-injury.

ROS may contribute to the cerebrovascular disturbances and changes in glucose metabolism that occur following TBI or ischemia[175;180]. Pyruvate dehydrogenase, a key enzyme in the glycolytic pathway, may be decomposed and inactivated by ROS[316], with lactate accumulation and reduced entry of pyruvate into the Krebs cycle. The

penthose phosphate shunt has a low activity in normal brain but may use significantly larger fractions of glucose when activated by oxidative stress on the glutathione pathway[20]. The improved rCBF after nitrone treatment is in accordance with improvements in rCBF with other ROS scavengers , such as vitamin E[40] and SOD[241], likely representing effects on the complex interplay between ROS, NO, peroxynitrite and other factors influencing vessel tone[17]. Superoxide anion and NO are known vasodilators[181] and NO causes modulation of tissue blood flow, platelet aggregation and microvascular permeability[56]. PBN was shown to increase CBF in anesthetized rats by reducing the breakdown of nitrous oxide[161]. However, superoxide and NO react to form peroxynitrite, that may act as a contractile agonist of cerebral artery smooth muscle cells[79]. Thus, a reduction of superoxide anion concentrations could improve rCBF through a decreased peroxynitrite formation and reduction of arterial wall tension. Reduced formation of peroxynitrite, a potent mitochondrial toxin, by nitrones may partly explain the present results.

4-HYDROXY BENZOIC ACID (4-HBA) AND MICRODIALYSIS FOR THE DETECTION OF ROS AFTER CONTROLLED CORTICAL CONTUSION INJURY (PAPER V)

In this Paper, the 4-hydroxybenzoic acid (4-HBA) trapping method was evaluated as a tool for ROS detection in conjunction with microdialysis in the controlled cortical contusion injury model.

In the first 30-min sample post-injury, there was, on average, a 250% increase in 3,4-DHBA formation in the severely injured animals that remained significantly increased relative to baseline and to the mildly traumatized and sham-operated animals for 90 min post-trauma. These results suggest increased ROS production after trauma. In contrast, the mildly traumatized animals showed a small, 100 % increase in 3,4-DHBA, statistically significant at 30 min post-injury only. The AUC calculation indicated that severely traumatized animals with central probe placement had significantly more formation of 3,4-DHBA as compared with sham and the mildly traumatized group with peripheral placement of the probe. The increase in ROS

formation confirmed that seen using the salicylate trapping technique in severely injured control animals.
The ipsilateral cerebral cortex surrounding the probe had a high total concentration of 4-HBA, distributed widely throughout the hemisphere. Tissue samples from the contralateral cortical area showed significantly lower 4-HBA concentrations ($p<0,05$). In extracerebral tissues, low 4-HBA levels were found in liver, lung, kidney and heart tissue. Occasional cortical samples in naive control animals showed a low concentration of 4-HBA (<10 ng/g) indicating an endogenous source of 4-HBA.

In conclusion, with this method we were able to detect a substantial increase in ROS formation after TBI. 4-HBA may be formed from tyrosine in the intestinal flora and is a normal constituent of human urine[310], making allergic reactions unlikely. No adverse reactions with 4-HBA are known to occur, suggesting that the method may have clinical applicability. However, because of the high concentrations of 4-HBA in ipsilateral brain tissue, careful toxicological analysis and testing is necessary before application in humans.
The formation of 3,4-DHBA is believed to occur specifically upon reaction with ROS and no enzymatic formation is known to take place. Previously, the aromatic compounds (salicylate, phenylalanine and 4-HBA) used for ROS detection were considered to specifically react with hydroxyl radicals but it has now been demonstrated that peroxynitrite may also cause oxidation of 4-HBA[27;137]. The increase in 3,4-DHBA after injury concurs with other methods studying ROS formation after TBI (see the Introduction section of this thesis) and the formation of only one adduct after reaction with ROS should be regarded as an advantage when compared with the other aromatic compounds. Here, we observed a baseline formation of 3,4-DHBA before injury that may be due to several factors such as metal ions in the dialysis fluid and in the microdialysis system. Moreover, the trauma caused by probe implantation will likely cause the release of iron from extravasated hemoglobin and the leakage of intracellular ions from damaged cells. Additionally, our results indicate that when performing microdialysis in the experimental setting, the exact location of the MD probe relative to the injury site is important and should be determined before injury.

PBN AND S-PBN PRE-TREATMENT AND ROS FORMATION AFTER CONTROLLED CORTICAL CONTUSION INJURY (PAPER VI)

In this Paper, the 4-HBA trapping method was used. The effects of PBN and S-PBN pre-treatment (30 mg/kg or 47 mg/kg i.v. 30 min before injury) on ROS formation was evaluated. Brain and plasma concentrations of PBN and S-PBN was measured in an attempt to evaluate TBI-induced changes in drug transport across the BBB.

As shown in Paper V, when compared with sham-operated controls, TBI was found to increase ROS formation. Pre-treatment with PBN significantly attenuated the increase of dialysate 3,4-DHBA (ANOVA; $F=12.0$, $p<0.005$); however, there was no statistically significant difference versus saline-treated animals at any individual time point. There was no statistically significant difference between PBN-treated rats and sham-injured controls. Pre-treatment with S-PBN also attenuated the 3,4-DHBA production (ANOVA; $F=5.4$, $p<0.05$) as compared with saline-treated animals. The S-PBN-treated animals showed lower 3,4-DHBA levels at 30 min after injury. Area under the curve (AUC) calculations are shown in Fig 6.

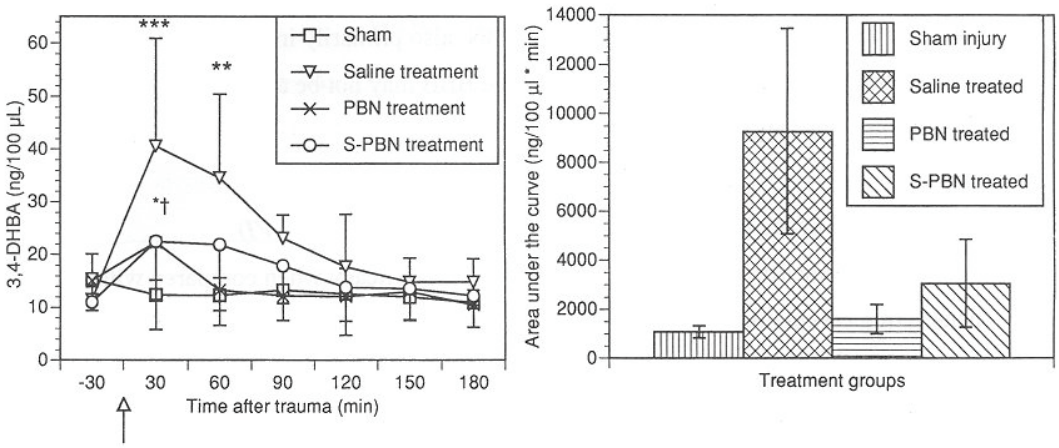


Fig.6. The concentrations of 3,4-DHBA in the dialysate after trauma or sham injury are shown to the left, where the arrow indicates time for trauma. The corresponding integrated area under the curve (AUC) from 30 min before and 180 min after trauma is shown to the right.

A significant difference was not detected between S-PBN treated and sham-injured control animals. In addition, there was no significant difference between PBN and S-PBN-treated rats.
Plasma and brain PBN concentrations remained high throughout the experiment[48]. At the end of the experiment, 210 min after injection, high PBN concentrations were found in all brain regions analyzed. No detectable S-PBN concentrations were found in plasma or any brain region at this time point. At 30 and 60 min post-injury, S-PBN concentrations were below the level of detection at both time-points in all brain regions, including the injured parietal cortices despite mean concentrations in plasma of 4125 and 230 ng/ml, respectively.

The results suggest that quenching of ROS contributes to the neuroprotective effects seen with PBN and S-PBN. S-PBN scavenged ROS formation in spite of undetectable concentrations in normal or traumatized brain tissue as early as 30 min post-injury and at a much lower plasma concentration in plasma than PBN. These results suggest that ROS formation at the blood-endothelial interface is an important event in the secondary injury cascade in TBI. However, an effect of nitrones on neurochemical cascades (e.g., calcium influx and mitochondrial disturbance), resulting in attenuation of secondary ROS formation is also possible. Additionally, the results presented here raise the question if PBN, in addition to S-PBN, also primarily influences factors at the blood-endothelial interface. Penetration of the BBB may not be a pre-requisite for a neuroprotective drug.

What goes on at the blood-endothelial interface? (Paper II, IV, VI)
BBB penetrating drugs are thought to have superior efficacy when compared with non-penetrating compounds[143;341]. However, compounds with poor BBB penetration such as NXY-059[193], tirilazad mesylate[125] and a peroxynitrite scavenger[130] have shown neuroprotective effects in experimental models of TBI. The endothelial cells (EC) contain the majority of the xanthine oxidase found in brain, and are exposed to the highest oxygen tension and to neutrophils with radical forming capacity[17;24;223;300]. EC also have high concentrations of mitochondria and polyunsaturated fatty acids[193;251]

that, under pathological situations, may increase the production of ROS[119;230]. Endothelial cells may be an important target for ROS-mediated injury[10] and contains SOD, glutathione and catalase for their protection[319]. Interaction between endothelial cells, cytokines, WBCs and adhesion molecules may both cause ROS formation and be influenced by ROS.

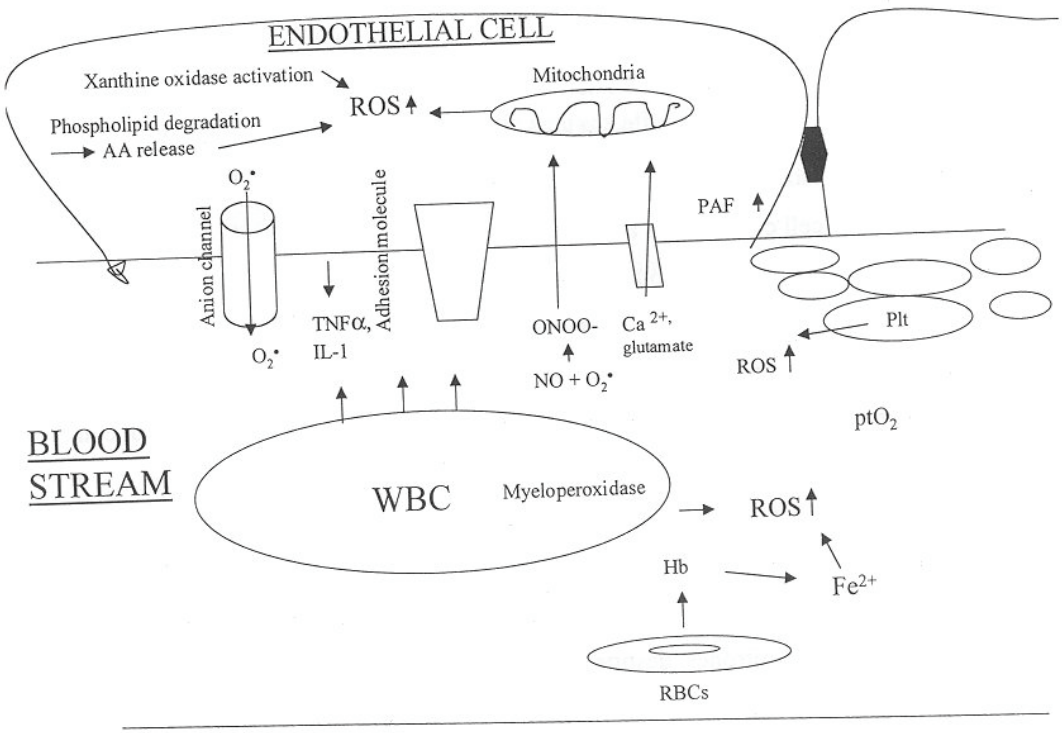


Fig. 7. Some possible mechanisms at the blood-endothelial interface in TBI that may contribute to the secondary injury cascade.
WBC- White blood cell, leucocyte; Plt- Platelets; Rbc-Erythrocytes; AA-Arachidonic acid; Hb- Hemoglobin. ONOO- - Peroxynitrite; HOCl – Hypochlorous acid.

WBCs, which contain important sources for free radicals and proteases, may cause adverse rheological and hemodynamic effects after TBI[8;64]. Although migration of WBCs into injured brain tissue does not occur to a significant extent until several

hours after injury, the increased adhesion of WBCs occurs early after TBI[142] and may cause release of ROS. ROS was also shown to influence neutrophil-endothelial cell interactions through upregulation of adhesion molecules such as CD11b, ICAM-1 and P-selectin[347]. In addition, *in vitro* work showed a release of TNFα and IL-1β from brain endothelial cells after percussive injury[117].

NO produced by eNOS may promote vasodilation and inhibit endothelial xanthine oxidase[158], reduce platelet aggregation[99] and decrease endothelial dysfunction, P-selectin expression and WBC adhesion[106]. Platelet activating factor (PAF) is synthesized in neurons and in injured brain, and may cause an increase in BBB permeability, vasoconstriction[177] or neurotoxicity[95]. PAF is involved in interactions between blood cell components and endothelial cells[34] and is induced by hydrogen peroxide[201]. Accumulation of platelets is observed after FPI[69] and platelets have a radical producing potential[253;295]. Finally, degradation of membrane phospholipids results in release of arachidonic acid[215;325] with ROS as a byproduct when acted upon by prostaglandin synthase. A consequence of phospholipid degradation is the opening of the BBB and induction of damage to vessels[180;334]. These factors could all contribute to endothelial cell damage and dysfunction, causing obstruction of brain capillaries and compromise substrate delivery to brain tissue in energetic stress after TBI. The marked reduction of ROS formation and attenuation of disturbances in rCBF and glucose uptake with nitrones, despite the poor BBB penetration of S-PBN, imply that the blood-endothelial interface is an important site for ROS production and ROS-mediated injury in TBI.

CONCLUSIONS

The present findings have led to the following conclusions:

- PBN pre-treatment improved morphological and functional outcome after controlled cortical contusion injury;

- PBN post-treatment improved morphological outcome and motor function, whereas S-PBN post-treatment improved cognitive and morphological outcome after fluid percussion injury;

- PBN increased the number of apoptotic neurons after moderate controlled cortical contusion injury despite the observed tissue sparing effects seen with PBN;

- PBN and S-PBN attenuated the changes in rCBF and glucose metabolism observed after fluid percussion injury;

- ROS formation were detected in a rodent model of controlled cortical contusion using the 4-hydroxybenzoic acid (4-HBA) trapping method; and

- PBN and S-PBN equally attenuated the ROS formation in a rodent model despite no detectable brain concentration of S-PBN.

In summary, these studies demonstrated that ROS are formed after TBI. Quenching of ROS may positively influence the secondary injury cascade and improve functional and morphological outcome in TBI. The 4-HBA trapping method may be useful clinically for the monitoring of ROS production in acute brain injury. Nitrone treatment may prove to be a valuable therapeutic concept for the treatment of traumatic brain injury in humans.

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Reference List

1. Abdel-Dayem HM, Abu-Judeh H, Kumar M, et al: SPECT brain perfusion abnormalities in mild or moderate traumatic brain injury. Clin.Nucl.Med. 23:309-317, 1998

2. Adams JH, Graham DI, Murray LS, et al: Diffuse axonal injury due to nonmissile head injury in humans: an analysis of 45 cases. Ann.Neurol 12:557-563, 1982

3. Alnemri ES, Livingston DJ, Nicholson DW, et al: Human ICE/CED-3 protease nomenclature. Cell 87:171, 1996

4. Althaus JS, Andrus PK, Williams CM, et al: The use of salicylate hydroxylation to detect hydroxyl radical generation in ischemic and traumatic brain injury. Reversal by tirilazad mesylate (U-74006F). Mol.Chem.Neuropathol. 20:147-162, 1993

5. Ames BN, Cathcart R, Schwiers E, et al: Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl.Acad.Sci.U.S.A 78:6858-6862, 1981

6. Amin AR, Vyas P, Attur M, et al: The mode of action of aspirin-like drugs: effect on inducible nitric oxide synthase. Proc.Natl.Acad.Sci.U.S.A 92:7926-7930, 1995

7. Andersen BJ, Marmarou A: Post-traumatic selective stimulation of glycolysis. Brain Res. 585:184-189, 1992

8. Anderson BO, Brown JM, Harken AH: Mechanisms of neutrophil-mediated tissue injury. J Surg.Res. 51:170-179, 1991

9. Anderson DE, Yuan XJ, Tseng CM, et al: Nitrone spin-traps block calcium channels and induce pulmonary artery relaxation independent of free radicals. Biochem.Biophys.Res.Commun. 193:878-885, 1993

10. Arroyo CM, Carmichael AJ, Bouscarel B, et al: Endothelial cells as a source of oxygen-free radicals. An ESR study. Free Radic.Res.Commun. 9:287-296, 1990

11. Atamna H, Paler-Martinez A, Ames BN: N-t-butyl hydroxylamine, a hydrolysis product of alpha-phenyl-N-t-butyl nitrone, is more potent in delaying senescence in human lung fibroblasts. J Biol.Chem. 275:6741-6748, 2000

12. Au AM, Chan PH, Fishman RA: Stimulation of phospholipase A2 activity by oxygen-derived free radicals in isolated brain capillaries. J.Cell Biochem. 27:449-453, 1985

13. Balasingam V, Tejada-Berges T, Wright E, et al: Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. J Neurosci 14:846-856, 1994

14. Bardt TF, Unterberg AW, Hartl R, et al: Monitoring of brain tissue PO2 in traumatic brain injury: effect of cerebral hypoxia on outcome. Acta Neurochir.Suppl (Wien.) 71:153-156, 1998

15. Becker DP, Vries JK, Sakalas R, et al: Early prognosis in head injury based on motor posturing, oculocephalic reflexes, and intracranial pressure. pp. 27-30. In: McLaurin.RL, ed.Head injuries.New York., Grune & Stratton, 1976.

16. Beckman JS: Peroxynitrite versus hydroxyl radical: the role of nitric oxide in superoxide-dependent cerebral injury. Ann.N.Y.Acad.Sci. 738:69-75, 1994

17. Beckman JS, Beckman TW, Chen J, et al: Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl.Acad.Sci.U.S.A 87:1620-1624, 1990

18. Beckman JS, Chen J, Ischiropoulos H, et al: Oxidative chemistry of peroxynitrite. Methods Enzymol. 233:229-240, 1994

19. Bederson JB, Pitts LH, Tsuji M, et al: Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke 17:472-476, 1986

20. Ben Yoseph O, Boxer PA, Ross BD: Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. J Neurochem. 66:2329-2337, 1996

21. Benzie IF, Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal.Biochem. 239:70-76, 1996

22. Bergsneider M, Hovda DA, Lee SM, et al: Dissociation of cerebral glucose metabolism and level of consciousness during the period of metabolic depression following human traumatic brain injury. J Neurotrauma 17:389-401, 2000

23. Bergsneider M, Hovda DA, Shalmon E, et al: Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study [see comments]. J Neurosurg. 86:241-251, 1997

24. Betz AL: Identification of hypoxanthine transport and xanthine oxidase activity in brain capillaries. J.Neurochem. 44:574-579, 1985

25. Binder LM: Persisting symptoms after mild head injury: a review of the postconcussive syndrome. J Clin.Exp.Neuropsychol. 8:323-346, 1986

26. Bindokas VP, Jordan J, Lee CC, et al: Superoxide production in rat hippocampal neurons: selective imaging with hydroethidine. J Neurosci 16:1324-1336, 1996

27. Bogdanov MB, Ferrante RJ, Mueller G, et al: Oxidative stress is attenuated in mice overexpressing BCL-2. Neurosci Lett. 262:33-36, 1999

28. Boucher BA, Hanes SD: Pharmacokinetic alterations after severe head injury. Clinical relevance [published erratum appears in Clin Pharmacokinet 2000 Apr;38(4):304]. Clin.Pharmacokinet. 35:209-221, 1998

29. Bouma GJ, Muizelaar JP, Choi SC, et al: Cerebral circulation and metabolism after severe traumatic brain injury: the elusive role of ischemia. J.Neurosurg. 75:685-693, 1991

30. Boveris A: Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria. Methods Enzymol. 105:429-435, 1984

31. Bramlett HM, Kraydieh S, Green EJ, et al: Temporal and regional patterns of axonal damage following traumatic brain injury: a beta-amyloid precursor protein immunocytochemical study in rats. J Neuropathol.Exp.Neurol 56:1132-1141, 1997

32. Brandeis R, Brandys Y, Yehuda S: The use of the Morris Water Maze in the study of memory and learning. Int.J.Neurosci. 48:29-69, 1989

33. Branston NM, Symon L, Crockard HA, et al: Relationship between the cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. Exp.Neurol 45:195-208, 1974

34. Braquet P, Bourgain R, Mencia-Huerta JM: Effect of platelet-activating factor on platelets and vascular endothelium. Semin.Thromb.Hemost. 15:184-196, 1989

35. Bredesen DE: Apoptosis: overview and signal transduction pathways. J Neurotrauma 17:801-810, 2000

36. Brooks N, Campsie L, Symington C, et al: The five year outcome of severe blunt head injury: a relative's view. J Neurol Neurosurg.Psychiatry 49:764-770, 1986

37. Buki A, Siman R, Trojanowski JQ, et al: The role of calpain-mediated spectrin proteolysis in traumatically induced axonal injury. J.Neuropathol.Exp.Neurol. 58:365-375, 1999

38. Bullock R, Zauner A, Myseros JS, et al: Evidence for prolonged release of excitatory amino acids in severe human head trauma. Relationship to clinical events. Ann.N.Y.Acad.Sci. 765:290-297, 1995

39. Burton GW, Joyce A, Ingold KU: First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet 2:327, 1982

40. Busto R, Yoshida S, Ginsberg MD, et al: Regional blood flow in compression-induced brain edema in rats: effect of dietary vitamin E. Ann.Neurol 15:441-448, 1984

41. Butterfield DA, Koppal T, Howard B, et al: Structural and functional changes in proteins induced by free radical- mediated oxidative stress and protective action of the antioxidants N-tert-butyl-alpha-phenylnitrone and vitamin E. Ann.N.Y.Acad.Sci. 854:448-462, 1998

42. Carney JM, Floyd RA: Protection against oxidative damage to CNS by alpha-phenyl-tert-butyl nitrone (PBN) and other spin-trapping agents: a novel series of nonlipid free radical scavengers. J.Mol.Neurosci. 3:47-57, 1991

43. Casadebaig F, Dupin JP, Gravier D, et al: Action of some salicylate derivatives on in vitro platelet aggregation. Inhibitory and inhibition antagonistic effects. Thromb.Res. 64:631-636, 1991

44. Ceballos G, Tuttle JB, Rubio R: Differential distribution of purine metabolizing enzymes between glia and neurons. J Neurochem. 62:1144-1153, 1994

45. Cecconi F: Apaf1 and the apoptotic machinery. Cell Death.Differ. 6:1087-1098, 1999

46. Chan PH: Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb.Blood Flow Metab 21:2-14, 2001

47. Chen GM, Bray TM, Janzen EG, et al: Excretion, metabolism and tissue distribution of a spin trapping agent, alpha-phenyl-N-tert-butyl-nitrone (PBN) in rats. Free Radic.Res.Commun. 9:317-323, 1990

48. Cheng HY, Liu T, Feuerstein G, et al: Distribution of spin-trapping compounds in rat blood and brain: in vivo microdialysis determination. Free Radic.Biol.Med. 14:243-250, 1993

49. Choi DW: At the scene of ischemic brain injury: is PARP a perp? [news]. Nat.Med. 3:1073-1074, 1997

50. Chopp M, Li Y, Jiang N: Increase in apoptosis and concomitant reduction of ischemic lesion volume and evidence for synaptogenesis after transient focal cerebral ischemia in rat treated with staurosporine. Brain Res. 828:197-201, 1999

51. Clark RS, Kochanek PM, Watkins SC, et al: Caspase-3 mediated neuronal death after traumatic brain injury in rats. J Neurochem. 74:740-753, 2000

52. Clarke DL, Johansson CB, Wilbertz J, et al: Generalized potential of adult neural stem cells. Science 288:1660-1663, 2000

53. Clifton GL, Lyeth BG, Jenkins LW, et al: Effect of D, alpha-tocopheryl succinate and polyethylene glycol on performance tests after fluid percussion brain injury. J Neurotrauma 6:71-81, 1989

54. Colicos MA, Dash PK: Apoptotic morphology of dentate gyrus granule cells following experimental cortical impact injury in rats: possible role in spatial memory deficits. Brain Res. 739:120-131, 1996

55. Conti AC, Raghupathi R, Trojanowski JQ, et al: Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. J.Neurosci. 18:5663-5672, 1998

56. Cooke JP, Dzau VJ: Nitric oxide synthase: role in the genesis of vascular disease. Annu.Rev.Med. 48:489-509, 1997

57. Cornford EM, Hyman S, Cornford ME, et al: Glut1 glucose transporter activity in human brain injury. J.Neurotrauma 13:523-536, 1996

58. Cortez SC, McIntosh TK, Noble LJ: Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. Brain Res. 482:271-282, 1989

59. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, et al: IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. J Neuroimmunol. 101:211-221, 1999

60. Damasio H, Grabowski T, Frank R, et al: The return of Phineas Gage: clues about the brain from the skull of a famous patient. Science 264:1102-1105, 1994

61. Dawson DA, Hallenbeck JM: Acute focal ischemia-induced alterations in MAP2 immunostaining: description of temporal changes and utilization as a marker for volumetric assessment of acute brain injury. J.Cereb.Blood Flow Metab 16:170-174, 1996

62. de Lange EC, Danhof M, de Boer AG, et al: Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. Brain Res.Brain Res.Rev. 25:27-49, 1997

63. Dejana E, Cerletti C, de Gaetano G: Interaction of salicylate and other non-steroidal anti-inflammatory drugs with aspirin on platelet and vascular cyclo-oxygenase activity. Thromb.Res.Suppl 4:153-159, 1983

64. del Zoppo GJ: Microvascular changes during cerebral ischemia and reperfusion. Cerebrovasc.Brain Metab Rev. 6:47-96, 1994

65. Denu JM, Tanner KG: Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. Biochemistry 37:5633-5642, 1998

66. Dewitt DS, Smith TG, Deyo DJ, et al: L-arginine and superoxide dismutase prevent or reverse cerebral hypoperfusion after fluid-percussion traumatic brain injury. J.Neurotrauma 14:223-233, 1997

67. Dietrich WD, Alonso O, Busto R, et al: Widespread metabolic depression and reduced somatosensory circuit activation following traumatic brain injury in rats. J.Neurotrauma 11:629-640, 1994

68. Dietrich WD, Alonso O, Busto R, et al: Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. Acta Neuropathol.(Berl) 87:250-258, 1994

69. Dietrich WD, Alonso O, Busto R, et al: Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: a double-label autoradiographic study in rats. J.Cereb.Blood Flow Metab 16:481-489, 1996

70. Dietrich WD, Alonso O, Busto R, et al: Posttraumatic cerebral ischemia after fluid percussion brain injury: an autoradiographic and histopathological study in rats. Neurosurgery 43:585-593, 1998

71. Doppenberg EM, Bullock R: Clinical neuro-protection trials in severe traumatic brain injury: lessons from previous studies. J Neurotrauma 14:71-80, 1997

72. Doppenberg EM, Rice MR, Di X, et al: Increased free radical production due to subdural hematoma in the rat: effect of increased inspired oxygen fraction. J.Neurotrauma 15:337-347, 1998

73. Dubey A, Forster MJ, Sohal RS: Effect of the spin-trapping compound N-tert-butyl-alpha-phenylnitrone on protein oxidation and life span. Arch.Biochem.Biophys. 324:249-254, 1995

74. Dubinsky JM, Kristal BS, Elizondo-Fournier M: An obligate role for oxygen in the early stages of glutamate-induced, delayed neuronal death. J Neurosci 15:7071-7078, 1995

75. Dugan LL, Sensi SL, Canzoniero LM, et al: Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. J Neurosci 15:6377-6388, 1995

76. Eguchi Y, Shimizu S, Tsujimoto Y: Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res. 57:1835-1840, 1997

77. Eldadah BA, Faden AI: Caspase pathways, neuronal apoptosis, and CNS injury. J Neurotrauma 17:811-829, 2000

78. Eliasson MJ, Sampei K, Mandir AS, et al: Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat.Med. 3:1089-1095, 1997

79. Elliott SJ, Lacey DJ, Chilian WM, et al: Peroxynitrite is a contractile agonist of cerebral artery smooth muscle cells. Am.J Physiol 275:H1585-H1591, 1998

80. Enblad P, Persson L: Impact on clinical outcome of secondary brain insults during the neurointensive care of patients with subarachnoid haemorrhage: a pilot study. J Neurol.Neurosurg.Psychiatry 62:512-516, 1997

81. Endres M, Wang ZQ, Namura S, et al: Ischemic brain injury is mediated by the activation of poly(ADP- ribose)polymerase. J Cereb.Blood Flow Metab 17:1143-1151, 1997

82. Estevez AG, Radi R, Barbeito L, et al: Peroxynitrite-induced cytotoxicity in PC12 cells: evidence for an apoptotic mechanism differentially modulated by neurotrophic factors. J Neurochem. 65:1543-1550, 1995

83. Fabian RH, DeWitt DS, Kent TA: The 21-aminosteroid U-74389G reduces cerebral superoxide anion concentration following fluid percussion injury of the brain. J.Neurotrauma 15:433-440, 1998

84. Faden AI, Demediuk P, Panter SS, et al: The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science 244:798-800, 1989

85. Feeney DM, Boyeson MG, Linn RT, et al: Responses to cortical injury: I. Methodology and local effects of contusions in the rat. Brain Res. 211:67-77, 1981

86. Ferger B, van Amsterdam C, Seyfried C, et al: Effects of alpha-phenyl-tert-butylnitrone and selegiline on hydroxyl free radicals in rat striatum produced by local application of glutamate. J.Neurochem. 70:276-280, 1998

87. Fineman I, Hovda DA, Smith M, et al: Concussive brain injury is associated with a prolonged accumulation of calcium: a 45Ca autoradiographic study. Brain Res. 624:94-102, 1993

88. Fink KB, Andrews LJ, Butler WE, et al: Reduction of post-traumatic brain injury and free radical production by inhibition of the caspase-1 cascade. Neuroscience 94:1213-1218, 1999

89. Fiskum G: Mitochondrial participation in ischemic and traumatic neural cell death. J Neurotrauma 17:843-855, 2000

90. Floyd RA, Carney J.M.: Neurodegeneration and neuroprotection in Parkinson´s disease. Academic Press Ltd, 1996, pp 79-90

91. Floyd RA, Carney JM: Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann.Neurol. 32 Suppl:S22-S27, 1992

92. Floyd RA, Henderson R, Watson JJ, et al: Use of salicylate with high pressure liquid chromatography and electrochemical detection (LCED) as a sensitive measure of hydroxyl free radicals in adriamycin treated rats. J Free Radic.Biol.Med. 2:13-18, 1986

93. Folbergrova J, He QP, Li PA, et al: The effect of alpha-phenyl-N-tert-butyl nitrone on bioenergetic state in substantia nigra following flurothyl-induced status epilepticus in rats. Neurosci.Lett. 266:121-124, 1999

94. Freeman BA, Crapo JD: Biology of disease: free radicals and tissue injury. Lab Invest 47:412-426, 1982

95. Frerichs KU, Feuerstein GZ: Platelet-activating factor--key mediator in neuroinjury? Cerebrovasc.Brain Metab Rev. 2:148-160, 1990

96. Fridovich I: Biological effects of the superoxide radical. Arch.Biochem.Biophys. 247:1-11, 1986

97. Fujimoto S, Kawakami N, Ohara A: Hydroxylation of phenylalanine and salicylate by stimulated polymorphonuclear leukocytes and the accelerating effect of glutathione on their hydroxylation. Biol.Pharm.Bull. 17:767-772, 1994

98. Fujisawa H, Landolt H, Bullock R: Patterns of increased glucose use following extracellular infusion of glutamate: an autoradiographic study. J Neurotrauma 13:245-254, 1996

99. Fukahori M, Ichimori K, Ishida H, et al: Nitric oxide reversibly suppresses xanthine oxidase activity. Free Radic.Res. 21:203-212, 1994

100. Gahm C, Holmin S, Mathiesen T: Temporal profiles and cellular sources of three nitric oxide synthase isoforms in the brain after experimental contusion. Neurosurgery 46:169-177, 2000

101. Gamaley IA, Klyubin IV: Roles of reactive oxygen species: signaling and regulation of cellular functions. Int.Rev.Cytol. 188:203-255, 1999

102. Gardner AM, Xu FH, Fady C, et al: Apoptotic vs. nonapoptotic cytotoxicity induced by hydrogen peroxide. Free Radic.Biol.Med. 22:73-83, 1997

103. Gavrieli Y, Sherman Y, Ben Sasson SA: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol. 119:493-501, 1992

104. Gennarelli TA: Animate models of human head injury. J Neurotrauma 11:357-368, 1994

105. Gerschman R, Gilbert, D. L, Dwyer, P, and Fenn, W. O. Oxygen poisoning and x-irradiation: A mechanisms in common. Science 119, 623-626. 1954.

106. Gidday JM, Beetsch JW, Park TS: Endogenous glutathione protects cerebral endothelial cells from traumatic injury. J.Neurotrauma 16:27-36, 1999

107. Gidday JM, Park TS, Shah AR, et al: Modulation of basal and postischemic leukocyte-endothelial adherence by nitric oxide. Stroke 29:1423-1429, 1998

108. Gido G, Cronberg T, Wieloch T: The effect of alpha-phenyl-tert-butyl nitrone (PBN) on free radical formation in transient focal ischaemia measured by microdialysis and 3,4-dihydroxybenzoate formation. Acta Physiol Scand. 168:277-285, 2000

109. Ginsberg MD, Zhao W, Alonso OF, et al: Uncoupling of local cerebral glucose metabolism and blood flow after acute fluid-percussion injury in rats. Am.J.Physiol 272:H2859-H2868, 1997

110. Globus MY, Alonso O, Dietrich WD, et al: Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J.Neurochem. 65:1704-1711, 1995

111. Golding EM, Robertson CS, Bryan RM, Jr.: The consequences of traumatic brain injury on cerebral blood flow and autoregulation: a review. Clin.Exp.Hypertens. 21:299-332, 1999

112. Goldsmith W: The physical processes producing head injuries., Head Injury Conf Proc. 1966, pp 350-382

113. Goodman JC, Gopinath SP, Valadka AB, et al: Lactic acid and amino acid fluctuations measured using microdialysis reflect physiological derangements in head injury. Acta Neurochir.Suppl (Wien.) 67:37-39, 1996

114. Goodman JC, Valadka AB, Gopinath SP, et al: Lactate and excitatory amino acids measured by microdialysis are decreased by pentobarbital coma in head-injured patients. J Neurotrauma 13:549-556, 1996

115. Goss JR, Taffe KM, Kochanek PM, et al: The antioxidant enzymes glutathione peroxidase and catalase increase following traumatic brain injury in the rat. Exp.Neurol 146:291-294, 1997

116. Gould TJ, Bickford PC: The effects of chronic treatment with N-tert-butyl-alpha-phenylnitrone on cerebellar noradrenergic receptor function in aged F344 rats. Brain Res. 660:333-336, 1994

117. Gourin CG, Shackford SR: Production of tumor necrosis factor-alpha and interleukin-1beta by human cerebral microvascular endothelium after percussive trauma. J Trauma 42:1101-1107, 1997

118. Graham DI, Adams JH, Doyle D: Ischaemic brain damage in fatal non-missile head injuries. J Neurol Sci. 39:213-234, 1978

119. Grammas P, Liu GJ, Wood K, et al: Anoxia/reoxygenation induces hydroxyl free radical formation in brain microvessels. Free Radic.Biol.Med. 14:553-557, 1993

120. Greenlund LJ, Deckwerth TL, Johnson EM, Jr.: Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death. Neuron 14:303-315, 1995

121. Grisar JM, Bolkenius FN, Petty MA, et al: 2,3-Dihydro-1-benzofuran-5-ols as analogues of alpha-tocopherol that inhibit in vitro and ex vivo lipid autoxidation and protect mice against central nervous system trauma. J Med.Chem. 38:453-458, 1995

122. Gutierrez E, Huang Y, Haglid K, et al: A new model for diffuse brain injury by rotational acceleration: I model, gross appearance, and astrocytosis. J Neurotrauma 18:247-257, 2001

123. Gutteridge JM: Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin.Chem. 41:1819-1828, 1995

124. Haber F and Weiss J. The catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc Lond Ser A (147), 332-351. 1934.

125. Hall ED: Inhibition of lipid peroxidation in central nervous system trauma and ischemia. J Neurol.Sci. 134 Suppl:79-83, 1995

126. Hall ED, Andrus PK, Yonkers PA: Brain hydroxyl radical generation in acute experimental head injury. J Neurochem. 60:588-594, 1993

127. Hall ED, Andrus PK, Yonkers PA, et al: Generation and detection of hydroxyl radical following experimental head injury. Ann.N.Y.Acad.Sci. 738:15-24, 1994

128. Hall ED, Andrus PK, Yonkers PA, et al: Generation and detection of hydroxyl radical following experimental head injury. Ann.N.Y.Acad.Sci. 738:15-24, 1994

129. Hall ED, Braughler JM, Yonkers PA, et al: U-78517F: a potent inhibitor of lipid peroxidation with activity in experimental brain injury and ischemia. J Pharmacol.Exp.Ther. 258:688-694, 1991

130. Hall ED, Kupina NC, Althaus JS: Peroxynitrite scavengers for the acute treatment of traumatic brain injury. Ann.N.Y.Acad.Sci. 890:462-468, 1999

131. Hall ED, Yonkers PA, McCall JM, et al: Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg. 68:456-461, 1988

132. Halliwell B: Ascorbic acid: hype, hoax, or healer? Am.J Clin.Nutr. 65:1891-1892, 1997

133. Halliwell B: Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic.Res. 31:261-272, 1999

134. Halliwell B, Gutteridge JM: The importance of free radicals and catalytic metal ions in human diseases. Mol.Aspects Med. 8:89-193, 1985

135. Halliwell B, Gutteridge JM: Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch.Biochem.Biophys. 246:501-514, 1986

136. Halliwell B, Gutteridge JM: Free Radicals in Biology and Medicine, ed Third edition. Oxford, UK: Clarendon Press, 1999,

137. Halliwell B, Kaur H: Hydroxylation of salicylate and phenylalanine as assays for hydroxyl radicals: a cautionary note visited for the third time. Free Radic.Res. 27:239-244, 1997

138. Halliwell B, Kaur H, Ingelman-Sundberg M: Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note [letter]. Free Radic.Biol.Med. 10:439-441, 1991

139. Hamm RJ, Temple MD, Pike BR, et al: The effect of postinjury administration of polyethylene glycol- conjugated superoxide dismutase (pegorgotein, Dismutec) or lidocaine on behavioral function following fluid-percussion brain injury in rats. J.Neurotrauma 13:325-332, 1996

140. Hamm RJ, Temple MD, Pike BR, et al: Working memory deficits following traumatic brain injury in the rat. J.Neurotrauma 13:317-323, 1996

141. Hartl R, Medary M, Ruge M, et al: Blood-brain barrier breakdown occurs early after traumatic brain injury and is not related to white blood cell adherence. Acta Neurochir.Suppl (Wien.) 70:240-242, 1997

142. Hartl R, Medary MB, Ruge M, et al: Early white blood cell dynamics after traumatic brain injury: effects on the cerebral microcirculation. J.Cereb.Blood Flow Metab 17:1210-1220, 1997

143. Haun SE, Kirsch JR, Helfaer MA, et al: Polyethylene glycol-conjugated superoxide dismutase fails to augment brain superoxide dismutase activity in piglets. Stroke 22:655-659, 1991

144. Hekmatpanah J, Hekmatpanah CR: Microvascular alterations following cerebral contusion in rats. Light, scanning, and electron microscope study. J.Neurosurg. 62:888-897, 1985

145. Hensley K, Pye QN, Maidt ML, et al: Interaction of alpha-phenyl-N-tert-butyl nitrone and alternative electron acceptors with complex I indicates a substrate reduction site upstream from the rotenone binding site. J.Neurochem. 71:2549-2557, 1998

146. Hicks R, Soares H, Smith D, et al: Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. Acta Neuropathol.(Berl) 91:236-246, 1996

147. Hicks RR, Smith DH, Lowenstein DH, et al: Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. J.Neurotrauma 10:405-414, 1993

148. Hicks RR, Smith DH, McIntosh TK: Temporal response and effects of excitatory amino acid antagonism on microtubule-associated protein 2 immunoreactivity following experimental brain injury in rats. Brain Res. 678:151-160, 1995

149. Hillered L, Ernster L: Respiratory activity of isolated rat brain mitochondria following in vitro exposure to oxygen radicals. J.Cereb.Blood Flow Metab 3:207-214, 1983

150. Hillered L, Persson L: Parabanic acid for monitoring of oxygen radical activity in the injured human brain. Neuroreport 6:1816-1820, 1995

151. Holbourn A.H. Mechanisms of head injury. Lancet (2), 438-441. 1943.

152. Holmin S, Mathiesen T, Shetye J, et al: Intracerebral inflammatory response to experimental brain contusion. Acta Neurochir.(Wien.) 132:110-119, 1995

153. Holmin S, Soderlund J, Biberfeld P, et al: Intracerebral inflammation after human brain contusion. Neurosurgery 42:291-298, 1998

154. Hovda DA, Lee SM, Smith ML, et al: The neurochemical and metabolic cascade following brain injury: moving from animal models to man. J.Neurotrauma 12:903-906, 1995

155. Hovda DA, Yoshino A, Kawamata T, et al: The increase in local cerebral glucose utilization following fluid percussion brain injury is prevented with kynurenic acid and is associated with an increase in calcium. Acta Neurochir.Suppl (Wien.) 51:331-333, 1990

156. Humayun MS, Presty SK, Lafrance ND, et al: Local cerebral glucose abnormalities in mild closed head injured patients with cognitive impairments. Nucl.Med.Commun. 10:335-344, 1989

157. Hyslop PA, Zhang Z, Pearson DV, et al: Measurement of striatal H2O2 by microdialysis following global forebrain ischemia and reperfusion in the rat: correlation with the cytotoxic potential of H2O2 in vitro. Brain Res. 671:181-186, 1995

158. Iadecola C: Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci 20:132-139, 1997

159. Ikeda Y, Brelsford KL, Ikeda K, et al: Oxygen-free radicals in traumatic brain oedema. Neurol Res. 11:213-216, 1989

160. Ikeda Y, Ikeda K, Long DM: Protective effect of the iron chelator deferoxamine on cold-induced brain edema. J Neurosurg. 71:233-238, 1989

161. Inanami O, Kuwabara M: alpha-Phenyl N-tert-butyl nitrone (PBN) increases the cortical cerebral blood flow by inhibiting the breakdown of nitric oxide in anesthetized rats. Free Radic.Res. 23:33-39, 1995

162. Inglis F, Kuroda Y, Bullock R: Glucose hypermetabolism after acute subdural hematoma is ameliorated by a competitive NMDA antagonist. J.Neurotrauma 9:75-84, 1992

163. Jorge RE, Robinson RG, Starkstein SE, et al: Influence of major depression on 1-year outcome in patients with traumatic brain injury. J Neurosurg. 81:726-733, 1994

164. Jorgensen OS, Hansen LI, Hoffman SW, et al: Synaptic remodeling and free radical formation after brain contusion injury in the rat. Exp.Neurol 144:326-338, 1997

165. Kaczorowski SL, Schiding JK, Toth CA, et al: Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. J Cereb.Blood Flow Metab 15:860-864, 1995

166. Katayama Y, Becker DP, Tamura T, et al: Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J.Neurosurg. 73:889-900, 1990

167. Katayama Y, Kawamata T, Tamura T, et al: Calcium-dependent glutamate release concomitant with massive potassium flux during cerebral ischemia in vivo. Brain Res. 558:136-140, 1991

168. Kawamata T, Katayama Y, Hovda DA, et al: Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. JCereb.Blood Flow Metab 12:12-24, 1992

169. Kawamata T, Katayama Y, Hovda DA, et al: Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. J.Cereb.Blood Flow Metab 12:12-24, 1992

170. Kawamata T, Katayama Y, Hovda DA, et al: Lactate accumulation following concussive brain injury: the role of ionic fluxes induced by excitatory amino acids. Brain Res. 674:196-204, 1995

171. Kawamata T, Katayama Y, Maeda T, et al: Antioxidant, OPC-14117, attenuates edema formation and behavioral deficits following cortical contusion in rats. Acta Neurochir.Suppl (Wien.) 70:191-193, 1997

172. Keller JN, Kindy MS, Holtsberg FW, et al: Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J.Neurosci. 18:687-697, 1998

173. Kerr JF, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br.J Cancer 26:239-257, 1972

174. Kiening KL, Unterberg AW, Bardt TF, et al: Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO2 versus jugular vein oxygen saturation. J Neurosurg. 85:751-757, 1996

175. Kirsch JR, Helfaer MA, Haun SE, et al: Polyethylene glycol-conjugated superoxide dismutase improves recovery of postischemic hypercapnic cerebral blood flow in piglets. Pediatr.Res. 34:530-537, 1993

176. Kochanek PM, Marion DW, Zhang W, et al: Severe controlled cortical impact in rats: assessment of cerebral edema, blood flow, and contusion volume. J.Neurotrauma 12:1015-1025, 1995

177. Kochanek PM, Nemoto EM, Melick JA, et al: Cerebrovascular and cerebrometabolic effects of intracarotid infused platelet-activating factor in rats. J Cereb.Blood Flow Metab 8:546-551, 1988

178. Kondo T, Kinouchi H, Kawase M, et al: Astroglial cells inhibit the increasing permeability of brain endothelial cell monolayer following hypoxia/reoxygenation. Neurosci Lett. 208:101-104, 1996

179. Kontos HA, Hess ML: Oxygen radicals and vascular damage. Adv.Exp.Med.Biol. 161:365-375, 1983

180. Kontos HA, Povlishock JT: Oxygen radicals in brain injury. Cent.Nerv.Syst.Trauma 3:257-263, 1986

181. Kontos HA, Wei EP: Superoxide production in experimental brain injury. J Neurosurg. 64:803-807, 1986

182. Kontos HA, Wei EP, Ellis EF, et al: Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats. Circ.Res. 57:142-151, 1985

183. Kooy NW, Royall JA, Ischiropoulos H, et al: Peroxynitrite-mediated oxidation of dihydrorhodamine 123. Free Radic.Biol.Med. 16:149-156, 1994

184. Kotake Y, Sang H, Miyajima T, et al: Inhibition of NF-kappaB, iNOS mRNA, COX2 mRNA, and COX catalytic activity by phenyl-N-tert-butylnitrone (PBN). Biochim.Biophys.Acta 1448:77-84, 1998

185. Kotamraju S, Konorev EA, Joseph J, et al: Doxorubicin-induced apoptosis in endothelial cells and cardiomyocytes is ameliorated by nitrone spin traps and ebselen. Role of reactive oxygen and nitrogen species. J Biol.Chem. 275:33585-33592, 2000

186. Kotapka MJ, Graham DI, Adams JH, et al: Hippocampal pathology in fatal human head injury without high intracranial pressure. J Neurotrauma 11:317-324, 1994

187. Koura SS, Doppenberg EM, Marmarou A, et al: Relationship between excitatory amino acid release and outcome after severe human head injury. Acta Neurochir.Suppl (Wien.) 71:244-246, 1998

188. Kraus JF, Nourjah P: The epidemiology of mild, uncomplicated brain injury. J Trauma 28:1637-1643, 1988

189. Krishnappa IK, Contant CF, Robertson CS: Regional changes in cerebral extracellular glucose and lactate concentrations following severe cortical impact injury and secondary ischemia in rats. J.Neurotrauma 16:213-224, 1999

190. Kruman II, Mattson MP: Pivotal role of mitochondrial calcium uptake in neural cell apoptosis and necrosis. J Neurochem. 72:529-540, 1999

191. Kulkarni M, Armstead WM: Superoxide generation links nociceptin/orphanin FQ (NOC/oFQ) release to impaired N-methyl-D-aspartate cerebrovasodilation after brain injury. Stroke 31:1990-1996, 2000

192. Kuroda S, Katsura K, Hillered L, et al: Delayed treatment with alpha-phenyl-N-tert-butyl nitrone (PBN) attenuates secondary mitochondrial dysfunction after transient focal cerebral ischemia in the rat. Neurobiol.Dis. 3:149-157, 1996

193. Kuroda S, Tsuchidate R, Smith ML, et al: Neuroprotective effects of a novel nitrone, NXY-059, after transient focal cerebral ischemia in the rat. J Cereb.Blood Flow Metab 19:778-787, 1999

194. Lagrange P, Romero IA, Minn A, et al: Transendothelial permeability changes induced by free radicals in an in vitro model of the blood-brain barrier. Free Radic.Biol.Med. 27:667-672, 1999

195. Leist M, Nicotera P: Apoptosis versus necrosis: the shape of neuronal cell death. Results Probl.Cell Differ. 24:105-135, 1998

196. Levin HS: Memory deficit after closed head injury. J Clin.Exp.Neuropsychol. 12:129-153, 1990

197. Levin HS: Prediction of recovery from traumatic brain injury. J Neurotrauma 12:913-922, 1995

198. Lewen A, Hillered L: Involvement of reactive oxygen species in membrane phospholipid breakdown and energy perturbation after traumatic brain injury in the rat. J.Neurotrauma 15:521-530, 1998

199. Lewen A, Li GL, Olsson Y, et al: Changes in microtubule-associated protein 2 and amyloid precursor protein immunoreactivity following traumatic brain injury in rat: influence of MK-801 treatment. Brain Res. 719:161-171, 1996

200. Lewen A, Matz P, Chan PH: Free radical pathways in CNS injury. J Neurotrauma 17:871-890, 2000

201. Lewis MS, Whatley RE, Cain P, et al: Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. J Clin.Invest 82:2045-2055, 1988

202. Lieberthal W, Menza SA, Levine JS: Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. Am.J Physiol 274:F315-F327, 1998

203. Liu D, Liu J, Wen J: Elevation of hydrogen peroxide after spinal cord injury detected by using the Fenton reaction. Free Radic.Biol.Med. 27:478-482, 1999

204. Liu PK, Hsu CY, Dizdaroglu M, et al: Damage, repair, and mutagenesis in nuclear genes after mouse forebrain ischemia-reperfusion. J Neurosci 16:6795-6806, 1996

205. Lockshin RA and Wilkins CM. Programmed cell death. II. Endocrine potentiation of the breakdown of the intersegmental muscles of silkmoths. J Insect Physiol (10), 643-649. 1964.

206. Loeffler JM, Ringer R, Hablutzel M, et al: The free radical scavenger alpha-phenyl-tert-butyl nitrone aggravates hippocampal apoptosis and learning deficits in experimental pneumococcal meningitis. J Infect.Dis. 183:247-252, 2001

207. Long DA, Ghosh K, Moore AN, et al: Deferoxamine improves spatial memory performance following experimental brain injury in rats. Brain Res. 717:109-117, 1996

208. Lowenstein DH, Thomas MJ, Smith DH, et al: Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J.Neurosci. 12:4846-4853, 1992

209. Lucignani G, Rossetti C, Ferrario P, et al: In vivo metabolism and kinetics of 99mTc-HMPAO. Eur.J.Nucl.Med. 16:249-255, 1990

210. Lundberg N. Acta Psychiatr Neurol Scand Suppl. 149(1), 1-193. 1960.

211. MacManus JP, Rasquinha I, Black MA, et al: Glutamate-treated rat cortical neuronal cultures die in a way different from the classical apoptosis induced by staurosporine. Exp.Cell Res. 233:310-320, 1997

212. Malis CD, Bonventre JV: Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. A model for post-ischemic and toxic mitochondrial damage. J.Biol.Chem. 261:14201-14208, 1986

213. Maples K, Yoshimoto T, Kanakaraj P, et al: NXY-059 prevents downregulation of Akt and release of cytochrome c after focal cerebral ischemia. Soc Neurosci Abstr.766-766, 2001.

214. Marklund N, Ostman B, Nalmo L, et al: Hypoxanthine, uric acid and allantoin as indicators of in vivo free radical reactions. Description of a HPLC method and human brain microdialysis data. Acta Neurochir.(Wien.) 142:1135-1141, 2000

215. Marklund N, Salci K, Lewen A, et al: Glycerol as a marker for post-traumatic membrane phospholipid degradation in rat brain. Neuroreport 8:1457-1461, 1997

216. Marklund SL, Holme E, Hellner L: Superoxide dismutase in extracellular fluids. Clin.Chim.Acta 126:41-51, 1982

217. Marshall JW, Duffin KJ, Green AR, et al: NXY-059, a free radical-trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. Stroke 32:190-198, 2001

218. Marshall LF, Maas AI, Marshall SB, et al: A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. J Neurosurg. 89:519-525, 1998

219. Marshall LF, Smith RW, Shapiro HM: The outcome with aggressive treatment in severe head injuries. Part I: the significance of intracranial pressure monitoring. J Neurosurg. 50:20-25, 1979

220. Marshall LF, Toole BM, Bowers SA: The National Traumatic Coma Data Bank. Part 2: Patients who talk and deteriorate: implications for treatment. J Neurosurg. 59:285-288, 1983

221. Martin NA, Patwardhan RV, Alexander MJ, et al: Characterization of cerebral hemodynamic phases following severe head trauma: hypoperfusion, hyperemia, and vasospasm. J Neurosurg. 87:9-19, 1997

222. Matsuo Y, Kihara T, Ikeda M, et al: Role of neutrophils in radical production during ischemia and reperfusion of the rat brain: effect of neutrophil depletion on extracellular ascorbyl radical formation. J.Cereb.Blood Flow Metab 15:941-947, 1995

223. McCord JM, Roy RS, Schaffer SW: Free radicals and myocardial ischemia. The role of xanthine oxidase. Adv.Myocardiol. 5:183-189, 1985

224. McDonald RP, Horsburgh KJ, Graham DI, et al: Mitochondrial DNA deletions in acute brain injury. Neuroreport 10:1875-1878, 1999

225. McIntosh TK, Juhler M, Wieloch T: Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. J.Neurotrauma 15:731-769, 1998

226. McIntosh TK, Saatman KE, Raghupathi R, et al: The Dorothy Russell Memorial Lecture. The molecular and cellular sequelae of experimental traumatic brain injury: pathogenetic mechanisms. Neuropathol.Appl.Neurobiol. 24:251-267, 1998

227. McIntosh TK, Smith DH, Meaney DF, et al: Neuropathological sequelae of traumatic brain injury: relationship to neurochemical and biomechanical mechanisms. Lab Invest 74:315-342, 1996

228. McIntosh TK, Thomas M, Smith D, et al: The novel 21-aminosteroid U74006F attenuates cerebral edema and improves survival after brain injury in the rat. J.Neurotrauma 9:33-46, 1992

229. McIntosh TK, Vink R, Noble L, et al: Traumatic brain injury in the rat: characterization of a lateral fluid- percussion model. Neuroscience 28:233-244, 1989

230. McKinney JS, Willoughby KA, Liang S, et al: Stretch-induced injury of cultured neuronal, glial, and endothelial cells. Effect of polyethylene glycol-conjugated superoxide dismutase. Stroke 27:934-940, 1996

231. McLean A, Jr., Temkin NR, Dikmen S, et al: The behavioral sequelae of head injury. J Clin.Neuropsychol. 5:361-376, 1983

232. Mecocci P, MacGarvey U, Beal MF: Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann.Neurol 36:747-751, 1994

233. Mickel HS, Vaishnav YN, Kempski O, et al: Breathing 100% oxygen after global brain ischemia in Mongolian Gerbils results in increased lipid peroxidation and increased mortality. Stroke 18:426-430, 1987

234. Mikawa S, Kinouchi H, Kamii H, et al: Attenuation of acute and chronic damage following traumatic brain injury in copper, zinc-superoxide dismutase transgenic mice. J.Neurosurg. 85:885-891, 1996

235. Miller JD: Head injury and brain ischaemia--implications for therapy. Br.J Anaesth. 57:120-130, 1985

236. Miller JD, Sweet RC, Narayan R, et al: Early insults to the injured brain. JAMA 240:439-442, 1978

237. Montgomery J, Ste-Marie L, Boismenu D, et al: Hydroxylation of aromatic compounds as indices of hydroxyl radical production: a cautionary note revisited. Free Radic.Biol.Med. 19:927-933, 1995

238. Morris.R. Development of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 4:47-60, 1984

239. Morrison B, III, Saatman KE, Meaney DF, et al: In vitro central nervous system models of mechanically induced trauma: a review. J Neurotrauma 15:911-928, 1998

240. Muir JK, Boerschel M, Ellis EF: Continuous monitoring of posttraumatic cerebral blood flow using laser- Doppler flowmetry. J.Neurotrauma 9:355-362, 1992

241. Muir JK, Tynan M, Caldwell R, et al: Superoxide dismutase improves posttraumatic cortical blood flow in rats. J.Neurotrauma 12:179-188, 1995

242. Muizelaar JP, Marmarou A, Young HF, et al: Improving the outcome of severe head injury with the oxygen radical scavenger polyethylene glycol-conjugated superoxide dismutase: a phase II trial [see comments]. J Neurosurg. 78:375-382, 1993

243. Murphy AN, Fiskum G, Beal MF: Mitochondria in neurodegeneration: bioenergetic function in cell life and death. J Cereb.Blood Flow Metab 19:231-245, 1999

244. Muzio M, Chinnaiyan AM, Kischkel FC, et al: FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. Cell 85:817-827, 1996

245. Newcomb JK, Zhao X, Pike BR, et al: Temporal profile of apoptotic-like changes in neurons and astrocytes following controlled cortical impact injury in the rat. Exp.Neurol 158:76-88, 1999

246. Nilsson P, Hillered L, Olsson Y, et al: Regional changes in interstitial K+ and Ca2+ levels following cortical compression contusion trauma in rats. J Cereb.Blood Flow Metab 13:183-192, 1993

247. Nilsson P, Hillered L, Ponten U, et al: Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. J.Cereb.Blood Flow Metab 10:631-637, 1990

248. Nilsson P, Laursen H, Hillered L, et al: Calcium movements in traumatic brain injury: the role of glutamate receptor-operated ion channels. J.Cereb.Blood Flow Metab 16:262-270, 1996

249. Nishio S, Yunoki M, Noguchi Y, et al: Detection of lipid peroxidation and hydroxyl radicals in brain contusion of rats. Acta Neurochir.Suppl (Wien.) 70:84-86, 1997

250. Okonkwo DO, Buki A, Siman R, et al: Cyclosporin A limits calcium-induced axonal damage following traumatic brain injury. Neuroreport 10:353-358, 1999

251. Oldendorf WH, Cornford ME, Brown WJ: The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. Ann.Neurol 1:409-417, 1977

252. Oliver CN, Starke-Reed PE, Stadtman ER, et al: Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. Proc.Natl.Acad.Sci.U.S.A 87:5144-5147, 1990

253. Orfeo T, Doherty JM, Adey G, et al: Sublethal percussion trauma in vitro causes a persisting derangement in the nonthrombogenic properties of brain endothelial cells. J Trauma 37:347-357, 1994

254. Park DS, Morris EJ, Stefanis L, et al: Multiple pathways of neuronal death induced by DNA-damaging agents, NGF deprivation, and oxidative stress. J Neurosci 18:830-840, 1998

255. Patel M, Day BJ, Crapo JD, et al: Requirement for superoxide in excitotoxic cell death. Neuron 16:345-355, 1996

256. Paulson OB, Strandgaard S, Edvinsson L: Cerebral autoregulation. Cerebrovasc.Brain Metab Rev. 2:161-192, 1990

257. Peeling J, Yan HJ, Chen SG, et al: Protective effects of free radical inhibitors in intracerebral hemorrhage in rat. Brain Res. 795:63-70, 1998

258. Pellegrini-Giampietro DE, Cherici G, Alesiani M, et al: Excitatory amino acid release and free radical formation may cooperate in the genesis of ischemia-induced neuronal damage. J.Neurosci. 10:1035-1041, 1990

259. Persson L, Hillered L: Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. J.Neurosurg. 76:72-80, 1992

260. Persson L, Valtysson J, Enblad P, et al: Neurochemical monitoring using intracerebral microdialysis in patients with subarachnoid hemorrhage [see comments]. J.Neurosurg. 84:606-616, 1996

261. Petito CK, Olarte JP, Roberts B, et al: Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol.Exp.Neurol 57:231-238, 1998

262. Petty MA, Poulet P, Haas A, et al: Reduction of traumatic brain injury-induced cerebral oedema by a free radical scavenger. Eur.J.Pharmacol. 307:149-155, 1996

263. Peuchen S, Bolanos JP, Heales SJ, et al: Interrelationships between astrocyte function, oxidative stress and antioxidant status within the central nervous system. Prog.Neurobiol. 52:261-281, 1997

264. Pierce JE, Smith DH, Trojanowski JQ, et al: Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. Neuroscience 87:359-369, 1998

265. Pogrebniak HW, Merino MJ, Hahn SM, et al: Spin trap salvage from endotoxemia: the role of cytokine down- regulation. Surgery 112:130-139, 1992

266. Pohl D, Bittigau P, Ishimaru MJ, et al: N-Methyl-D-aspartate antagonists and apoptotic cell death triggered by head trauma in developing rat brain. Proc Natl.Acad.Sci.U.S.A 96:2508-2513, 1999

267. Pontén U, Persson L, Hillered L: Traumatiska hjärnskador., in Widén L (ed): En bok om hjärnan. 1995, pp 303-322

268. Portera-Cailliau C, Price DL, Martin LJ: Excitotoxic neuronal death in the immature brain is an apoptosis- necrosis morphological continuum. J Comp Neurol 378:70-87, 1997

269. Prado R, Watson BD, Kuluz J, et al: Endothelium-derived nitric oxide synthase inhibition. Effects on cerebral blood flow, pial artery diameter, and vascular morphology in rats. Stroke 23:1118-1123, 1992

270. Racker E, Johnson JH, Blackwell MT: The role of ATPase in glycolysis of Ehrlich ascites tumor cells. J.Biol.Chem. 258:3702-3705, 1983

271. Raghupathi R, Graham DI, McIntosh TK: Apoptosis after traumatic brain injury. J Neurotrauma 17:927-938, 2000

272. Rao AM, Dogan A, Hatcher JF, et al: Fluorometric assay of nitrite and nitrate in brain tissue after traumatic brain injury and cerebral ischemia. Brain Res. 793:265-270, 1998

273. Reiter RJ: Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J 9:526-533, 1995

274. Rink A, Fung KM, Trojanowski JQ, et al: Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. Am.J Pathol. 147:1575-1583, 1995

275. Robertson CS, Grossman RG, Goodman JC, et al: The predictive value of cerebral anaerobic metabolism with cerebral infarction after head injury. J Neurosurg. 67:361-368, 1987

276. Robertson CS, Narayan RK, Gokaslan ZL, et al: Cerebral arteriovenous oxygen difference as an estimate of cerebral blood flow in comatose patients. J Neurosurg. 70:222-230, 1989

277. Robinson RG, Szetela B: Mood change following left hemispheric brain injury. Ann.Neurol 9:447-453, 1981

278. Rockswold GL, Pheley PJ: Patients who talk and deteriorate. Ann.Emerg.Med. 22:1004-1007, 1993

279. Romner B, Bellner J, Kongstad P, et al: Elevated transcranial Doppler flow velocities after severe head injury: cerebral vasospasm or hyperemia? J Neurosurg. 85:90-97, 1996

280. Romner B, Ingebrigtsen T, Kock-Jensen C: [Scandinavian guidelines for management of head injuries. Evidence- based management of minimal, mild and moderate head injuries]. Lakartidningen 97:3186-3192, 2000

281. Rose IA, Warms JV, O'Connell EL: Role of inorganic phosphate in stimulating the glucose utilization of human red blood cells. Biochem.Biophys.Res.Commun. 15:33-37, 1964

282. Rostworowski M, Balasingam V, Chabot S, et al: Astrogliosis in the neonatal and adult murine brain post-trauma: elevation of inflammatory cytokines and the lack of requirement for endogenous interferon-gamma. J Neurosci 17:3664-3674, 1997

283. Salazar JJ, Van Houten B: Preferential mitochondrial DNA injury caused by glucose oxidase as a steady generator of hydrogen peroxide in human fibroblasts. Mutat.Res. 385:139-149, 1997

284. Sanada T, Nakamura T, Nishimura MC, et al: Effect of U74006F on neurologic function and brain edema after fluid percussion injury in rats. J Neurotrauma 10:65-71, 1993

285. Sarrafzadeh AS, Thomale UW, Kroppenstedt SN, et al: Neuroprotective effect of melatonin on cortical impact injury in the rat. Acta Neurochir.(Wien.) 142:1293-1299, 2000

286. Sato I, Kaji K, Murota S: Age related decline in cytokine induced nitric oxide synthase activation and apoptosis in cultured endothelial cells: minimal involvement of nitric oxide in the apoptosis. Mech.Ageing Dev. 81:27-36, 1995

287. Schinder AF, Olson EC, Spitzer NC, et al: Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. J.Neurosci 16:6125-6133, 1996

288. Schmidt RH, Grady MS: Regional patterns of blood-brain barrier breakdown following central and lateral fluid percussion injury in rodents. J.Neurotrauma 10:415-430, 1993

289. Schoettle RJ, Kochanek PM, Magargee MJ, et al: Early polymorphonuclear leukocyte accumulation correlates with the development of posttraumatic cerebral edema in rats. J Neurotrauma 7:207-217, 1990

290. Schroder ML, Muizelaar JP, Kuta AJ, et al: Thresholds for cerebral ischemia after severe head injury: relationship with late CT findings and outcome. J Neurotrauma 13:17-23, 1996

291. Schulz JB, Henshaw DR, Siwek D, et al: Involvement of free radicals in excitotoxicity in vivo. J.Neurochem. 64:2239-2247, 1995

292. Schulz JB, Matthews RT, Beal MF: Role of nitric oxide in neurodegenerative diseases. Curr.Opin.Neurol 8:480-486, 1995

293. Schulz JB, Matthews RT, Jenkins BG, et al: Improved therapeutic window for treatment of histotoxic hypoxia with a free radical spin trap. J.Cereb.Blood Flow Metab 15:948-952, 1995

294. Schwartz LM, Osborne BA: Programmed cell death, apoptosis and killer genes. Immunol.Today 14:582-590, 1993

295. Seligmann C, Schimmer M, Leitsch T, et al: A thrombocyte-induced myocardial dysfunction in the ischemic and reperfused guinea pig heart is mediated by reactive oxygen species. Free Radic.Biol.Med. 29:1244-1251, 2000

296. Sen S, Goldman H, Morehead M, et al: alpha-Phenyl-tert-butyl-nitrone inhibits free radical release in brain concussion. Free Radic.Biol.Med. 16:685-691, 1994

297. Sheng H, Bart RD, Oury TD, et al: Mice overexpressing extracellular superoxide dismutase have increased resistance to focal cerebral ischemia. Neuroscience 88:185-191, 1999

298. Sheng H, Brady TC, Pearlstein RD, et al: Extracellular superoxide dismutase deficiency worsens outcome from focal cerebral ischemia in the mouse. Neurosci Lett. 267:13-16, 1999

299. Shohami E, Beit-Yannai E, Horowitz M, et al: Oxidative stress in closed-head injury: brain antioxidant capacity as an indicator of functional outcome. J.Cereb.Blood Flow Metab 17:1007-1019, 1997

300. Siesjo BK: Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. J Neurosurg. 77:337-354, 1992

301. Siesjo BK, Agardh CD, Bengtsson F: Free radicals and brain damage. Cerebrovasc.Brain Metab Rev. 1:165-211, 1989

302. Skoglosa Y, Lewen A, Takei N, et al: Regulation of pituitary adenylate cyclase activating polypeptide and its receptor type 1 after traumatic brain injury: comparison with brain- derived neurotrophic factor and the induction of neuronal cell death. Neuroscience 90:235-247, 1999

303. Slee EA, Adrain C, Martin SJ: Serial killers: ordering caspase activation events in apoptosis. Cell Death.Differ. 6:1067-1074, 1999

304. Smith DH, Chen XH, Pierce JE, et al: Progressive atrophy and neuron death for one year following brain trauma in the rat. J.Neurotrauma 14:715-727, 1997

305. Smith DH, Okiyama K, Thomas MJ, et al: Evaluation of memory dysfunction following experimental brain injury using the Morris water maze. J.Neurotrauma 8:259-269, 1991

306. Smith SL, Andrus PK, Zhang JR, et al: Direct measurement of hydroxyl radicals, lipid peroxidation, and blood- brain barrier disruption following unilateral cortical impact head injury in the rat. J.Neurotrauma 11:393-404, 1994

307. Soares HD, Hicks RR, Smith D, et al: Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. J Neurosci 15:8223-8233, 1995

308. Soares HD, Thomas M, Cloherty K, et al: Development of prolonged focal cerebral edema and regional cation changes following experimental brain injury in the rat. J Neurochem. 58:1845-1852, 1992

309. Sorg O, Horn TF, Yu N, et al: Inhibition of astrocyte glutamate uptake by reactive oxygen species: role of antioxidant enzymes. Mol.Med. 3:431-440, 1997

310. Ste-Marie L, Boismenu D, Vachon L, et al: Evaluation of sodium 4-hydroxybenzoate as an hydroxyl radical trap using gas chromatography-mass spectrometry and high-performance liquid chromatography with electrochemical detection. Anal.Biochem. 241:67-74, 1996

311. Stover JF, Schoning B, Beyer TF, et al: Temporal profile of cerebrospinal fluid glutamate, interleukin-6, and tumor necrosis factor-alpha in relation to brain edema and contusion following controlled cortical impact injury in rats. Neurosci Lett. 288:25-28, 2000

312. Stålhammar D. Experimental models of head injury. Acta Neurochir.Suppl (Wien.) 36, 33-46. 1986.

313. Sun JZ, Kaur H, Halliwell B, et al: Use of aromatic hydroxylation of phenylalanine to measure production of hydroxyl radicals after myocardial ischemia in vivo. Direct evidence for a pathogenetic role of the hydroxyl radical in myocardial stunning. Circ.Res. 73:534-549, 1993

314. Sutton RL, Lescaudron L, Stein DG: Unilateral cortical contusion injury in the rat: vascular disruption and temporal development of cortical necrosis. J.Neurotrauma 10:135-149, 1993

315. Szabo C: DNA strand breakage and activation of poly-ADP ribosyltransferase: a cytotoxic pathway triggered by peroxynitrite. Free Radic.Biol.Med. 21:855-869, 1996

316. Tabatabaie T, Potts JD, Floyd RA: Reactive oxygen species-mediated inactivation of pyruvate dehydrogenase. Arch.Biochem.Biophys. 336:290-296, 1996

317. Tanaka J, Toku K, Zhang B, et al: Astrocytes prevent neuronal death induced by reactive oxygen and nitrogen species. Glia 28:85-96, 1999

318. Tanaka K, Gotoh F, Gomi S, et al: Inhibition of nitric oxide synthesis induces a significant reduction in local cerebral blood flow in the rat. Neurosci Lett. 127:129-132, 1991

319. Tayarani I, Chaudiere J, Lefauconnier JM, et al: Enzymatic protection against peroxidative damage in isolated brain capillaries. J Neurochem. 48:1399-1402, 1987

320. Thiels E, Urban NN, Gonzalez-Burgos GR, et al: Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase. J Neurosci 20:7631-7639, 2000

321. Thornberry NA, Lazebnik Y: Caspases: enemies within. Science 281:1312-1316, 1998

322. Treves A, Rolls ET: Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. Hippocampus 2:189-199, 1992

323. Trounce I, Byrne E, Marzuki S: Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. Lancet 1:637-639, 1989

324. Tyurin VA, Tyurina YY, Borisenko GG, et al: Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. J Neurochem. 75:2178-2189, 2000

325. Unterberg A, Wahl M, Baethmann A: Effects of free radicals on permeability and vasomotor response of cerebral vessels. Acta Neuropathol.(Berl) 76:238-244, 1988

326. Vagnozzi R, Marmarou A, Tavazzi B, et al: Changes of cerebral energy metabolism and lipid peroxidation in rats leading to mitochondrial dysfunction after diffuse brain injury. J Neurotrauma 16:903-913, 1999

327. van Santbrink H, Maas AI, Avezaat CJ: Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. Neurosurgery 38:21-31, 1996

328. Vespa P, Prins M, Ronne-Engstrom E, et al: Increase in extracellular glutamate caused by reduced cerebral perfusion pressure and seizures after human traumatic brain injury: a microdialysis study. J.Neurosurg. 89:971-982, 1998

329. Vespa PM, Nenov V, Nuwer MR: Continuous EEG monitoring in the intensive care unit: early findings and clinical efficacy. J Clin.Neurophysiol. 16:1-13, 1999

330. Wada K, Alonso OF, Busto R, et al: Early treatment with a novel inhibitor of lipid peroxidation (LY341122) improves histopathological outcome after moderate fluid percussion brain injury in rats. Neurosurgery 45:601-608, 1999

331. Walker PR, Kokileva L, LeBlanc J, et al: Detection of the initial stages of DNA fragmentation in apoptosis. Biotechniques 15:1032-1040, 1993

332. Warme PE, Bergstrom R, Persson L: Neurosurgical intensive care improves outcome after severe head injury. Acta Neurochir.(Wien.) 110:57-64, 1991

333. Wei EP, Christman CW, Kontos HA, et al: Effects of oxygen radicals on cerebral arterioles. Am.J Physiol 248:H157-H162, 1985

334. Wei EP, Kontos HA, Christman CW, et al: Superoxide generation and reversal of acetylcholine-induced cerebral arteriolar dilation after acute hypertension. Circ.Res. 57:781-787, 1985

335. Whalen MJ, Clark RS, Dixon CE, et al: Reduction of cognitive and motor deficits after traumatic brain injury in mice deficient in poly(ADP-ribose) polymerase. J Cereb.Blood Flow Metab 19:835-842, 1999

336. Whisler RL, Goyette MA, Grants IS, et al: Sublethal levels of oxidant stress stimulate multiple serine/threonine kinases and suppress protein phosphatases in Jurkat T cells. Arch.Biochem.Biophys. 319:23-35, 1995

337. Wilde GJ, Pringle AK, Wright P, et al: Differential vulnerability of the CA1 and CA3 subfields of the hippocampus to superoxide and hydroxyl radicals in vitro. J Neurochem. 69:883-886, 1997

338. Wilkins, RH. Neurosurgical classic-XVII. J Neurosurgery (March), 240-244. 1964.

339. Wyllie AH, Morris RG, Smith AL, et al: Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol. 142:67-77, 1984

340. Xiong Y, Gu Q, Peterson PL, et al: Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. J.Neurotrauma 14:23-34, 1997

341. Xue D, Slivka A, Buchan AM: Tirilazad reduces cortical infarction after transient but not permanent focal cerebral ischemia in rats. Stroke 23:894-899, 1992

342. Yakovlev AG, Knoblach SM, Fan L, et al: Activation of CPP32-like caspases contributes to neuronal apoptosis and neurological dysfunction after traumatic brain injury. J Neurosci 17:7415-7424, 1997

343. Yamakami I, Yamaura A, Isobe K: Types of traumatic brain injury and regional cerebral blood flow assessed by 99mTc-HMPAO SPECT. Neurol.Med.Chir (Tokyo) 33:7-12, 1993

344. Yang Y, Li Q, Shuaib A: Neuroprotection by 2-h postischemia administration of two free radical scavengers, alpha-phenyl-n-tert-butyl-nitrone (PBN) and N-tert-butyl-(2- sulfophenyl)-nitrone (S-PBN), in rats subjected to focal embolic cerebral ischemia. Exp.Neurol. 163:39-45, 2000

345. Yoshida K, Burton GF, Young H, et al: Brain levels of polyethylene glycol-conjugated superoxide dismutase following fluid percussion brain injury in rats. J Neurotrauma 9:85-92, 1992

346. Yoshida S, Abe K, Busto R, et al: Influence of transient ischemia on lipid-soluble antioxidants, free fatty acids and energy metabolites in rat brain. Brain Res. 245:307-316, 1982

347. Yoshikawa T, Yoshida N, Konda M: Active oxygen species in neutrophil-endothelial cell interactions, in Packer L HM, Yoshikawa T (eds): Free radicals in brain physiology and disorders. San Diego, USA: Academic Press, inc., 1996, pp 45-50

348. Young B, Runge JW, Waxman KS, et al: Effects of pegorgotein on neurologic outcome of patients with severe head injury. A multicenter, randomized controlled trial. JAMA 276:538-543, 1996

349. Yu N, Maciejewski-Lenoir D, Bloom FE, et al: Tumor necrosis factor-alpha and interleukin-1 alpha enhance glucose utilization by astrocytes: involvement of phospholipase A2. Mol.Pharmacol. 48:550-558, 1995

350. Zhang D, Dhillon HS, Mattson MP, et al: Immunohistochemical detection of the lipid peroxidation product 4- hydroxynonenal after experimental brain injury in the rat. Neurosci Lett. 272:57-61, 1999

351. Zhao Q, Pahlmark K, Smith ML, et al: Delayed treatment with the spin trap alpha-phenyl-N-tert-butyl nitrone (PBN) reduces infarct size following transient middle cerebral artery occlusion in rats. Acta Physiol Scand. 152:349-350, 1994

352. Zipfel GJ, Babcock DJ, Lee JM, et al: Neuronal apoptosis after CNS injury: the roles of glutamate and calcium. J Neurotrauma 17:857-869, 2000

353. Zola-Morgan S, Squire LR: Neuroanatomy of memory. Annu.Rev.Neurosci 16:547-563, 1993