We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

7,000
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 10

Metabolic Responses and Profiling of Bioorganic Phosphates and Phosphate Metabolites in Traumatic Brain Injury

Noam Naphatali Tal, Tesla Yudhistira, Woo Hyun Lee, Youngsam Kim and David G. Churchill

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75745

Abstract

This chapter constitutes a review of the recent literature on metabolic response and profiling of bioorganic phosphates and phosphate metabolites in disease related to traumatic brain injury (TBI). In this report we emphasize the emerging role of advanced imaging techniques in both the translational research of TBI biology and in the development of new modalities for the diagnosis and therapy of TBI-related diseases. To date, several neuroimaging techniques have been used for assessing phosphate metabolites related to TBI. These techniques include ^{31}P-MRI/MRS imaging, magnetic resonance imaging, and incorporation of phosphate derivative hydrogels, all of which are of particular interest in identifying TBI. These advanced neuroimaging techniques are currently under investigation in an attempt to optimize properties for therapeutics purposes. In addition, this chapter also discusses the role of endogenous and exogenous phosphates related to TBI. TBI imaging is a rapidly evolving field, and a number of the recommendations presented will be updated in the future to reflect the advances in medical knowledge.

Keywords: phosphate, TBI, molecular imaging, phosphorylation, brain edema, MRI contrast agents

1. Introduction

As progress of medical science/technology and imaging accelerates into the future, this work is intended as an important review regarding the related chemistry and biochemistry

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

of traumatic brain injury (TBI). While some pertinent reviews have also appeared [1], we review what is known regarding phosphate chemistry. This is of critical importance for future researchers, and relates to brain-related injury, especially TBI. We sought to cover phosphates and phosphorylation in this context. From a database search, a list of keywords (phosphate, phosphonate, phosphorylation, traumatic brain injury, and probe, imaging, or sensor) and approximately 35 references have been acquired (ISI Web of Science, accessed in 2017). [1–35]. Instrumental techniques are also critically important and certain physical techniques are introduced and described as well (**Figures 1** and **2**). Reviews of biological phosphate imaging have emerged in the literature [36–39] and a combination of clinical and research aspects are presented. In addition, critically important phosphate species, probes, proteins, and related medicinal molecules are illustrated (**Figures 3–6**).

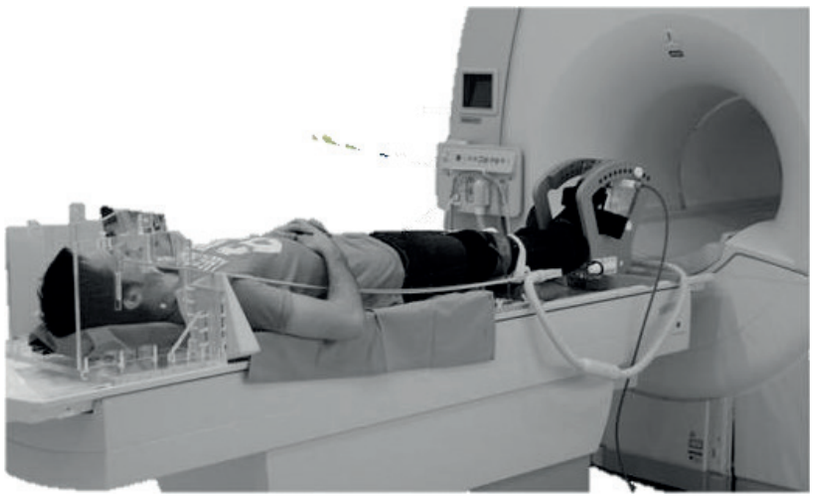

Figure 1. Dynamic 31P-magnetic resonance spectroscopy (http://www.mrtm.ethz.ch/research/mr-spectroscopy/physiological-projects/muscle-physiology.html).

Figure 2. Instruments used in the scientific laboratory such as the multinuclear NMR spectrometer (left), high-resolution mass spectrometer (middle), and fluorimeter (right). (Photos acquired at KAIST (Daejeon, Korea); high-resolutionmass spectrometer photo taken from kara.kaist.ac.kr.).

http://dx.doi.org/10.5772/intechopen.75745

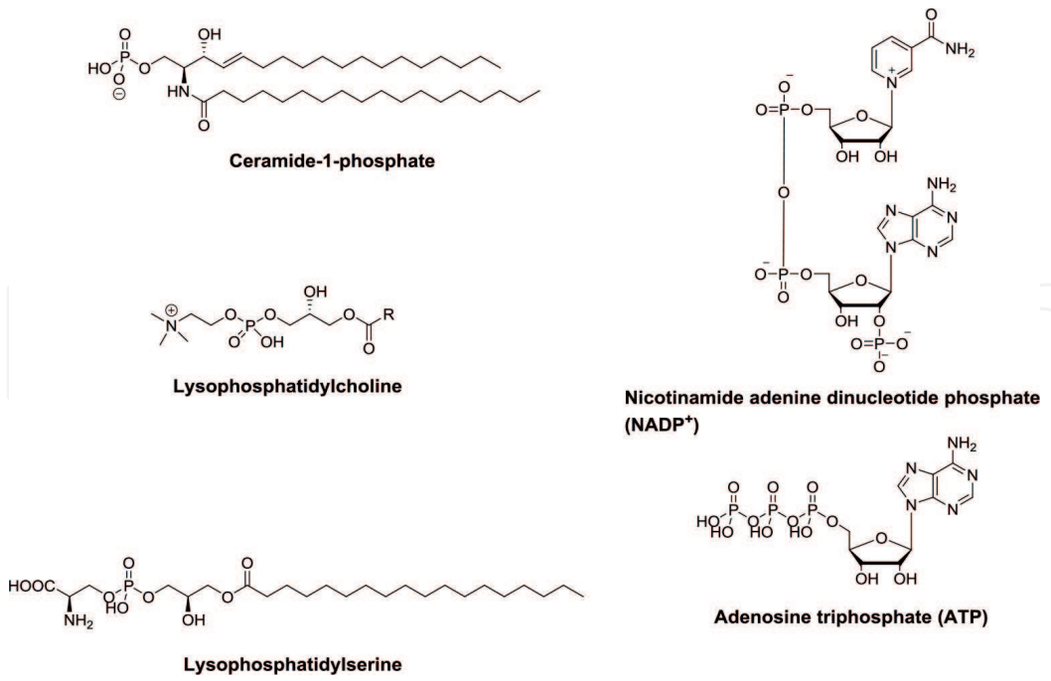

Figure 3. Phosphates under discussion in this review.

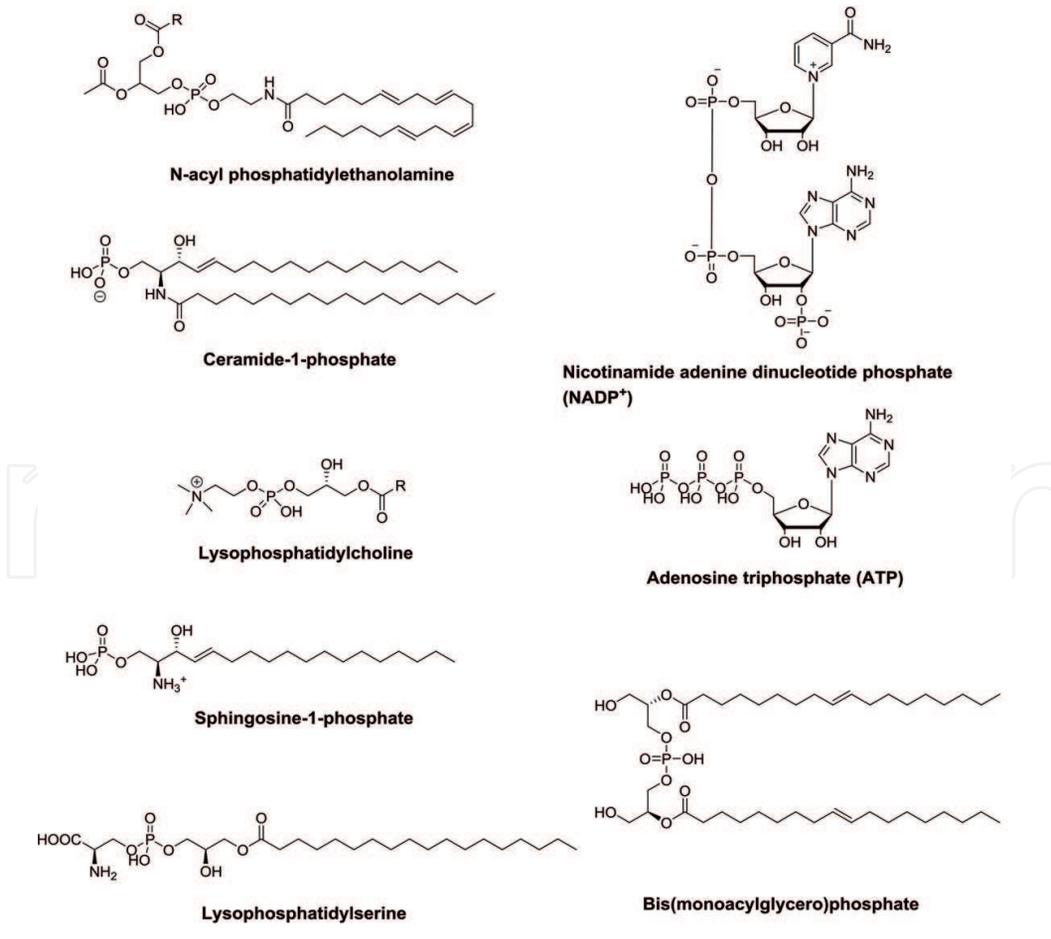

Figure 4. Phosphates under discussion in this review.

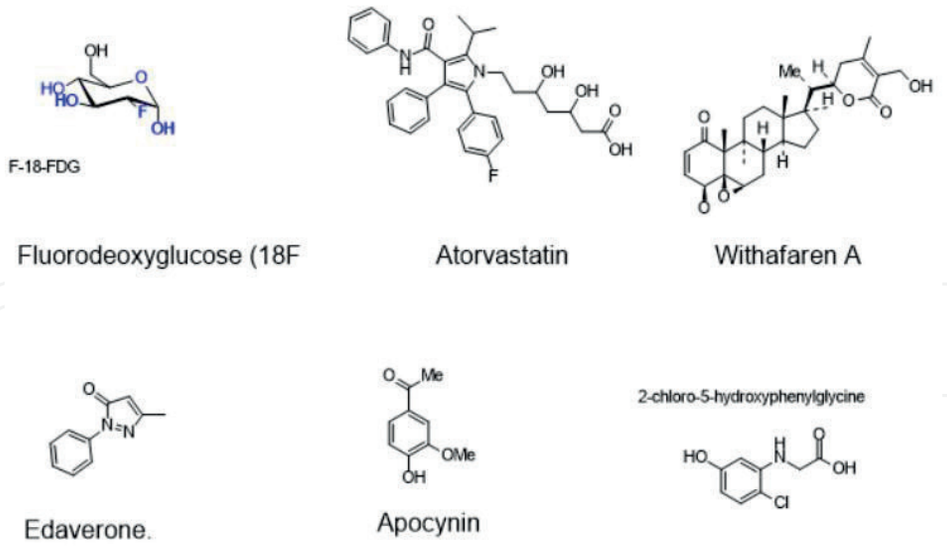

Figure 5. Therapeutic agents studied in the context of TBI and related studies. The nicotinamide adenine dinucleotide phosphate (NADP) oxidase inhibitor.

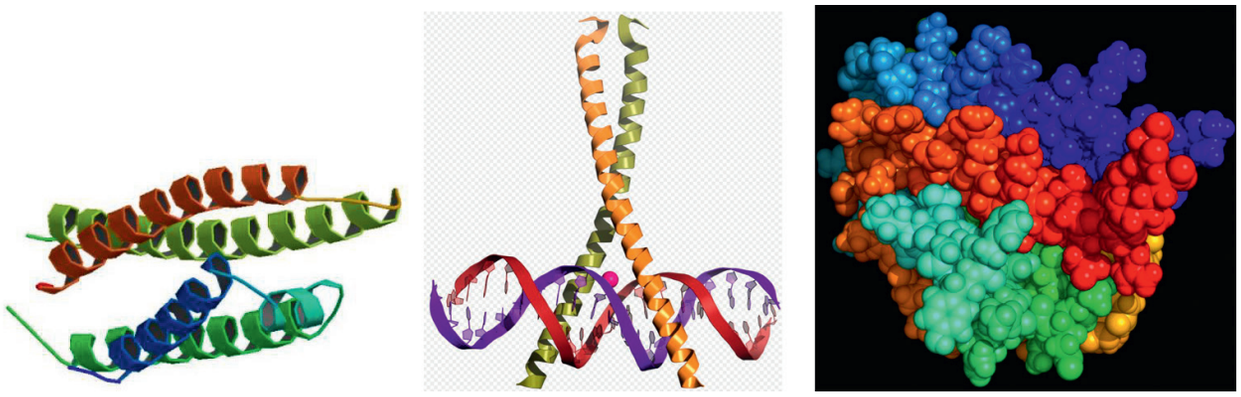

Figure 6. Some of the important proteins under discussion including apolipoprotein (APOE), the CREB protein, etc. (Copied without permission from the Internet, accessed in September 2017, Wikipedia.). CREB (top) is a transcription factor capable of binding DNA (bottom) and regulating gene expression.

2. TBI introduction

According to the US Centers for Disease Control and Prevention (CDC), every year in the United States approximately 1.7 million individuals receive an injury classified as a TBI), and 52,000 of these cases led to death [40]. TBI can be defined as alterations in brain functions and brain metabolism due to head collision with a stationary or moving object, or striking of a physical subject or coupling of an external mechanical force (e.g., g-force, blast shockwave) with the head [41–44]. Research has revealed that TBI can be associated with a variety of outcomes, from mild shock upon a single impact, to developing chronic traumatic encephalopathy (CTE) at a later time, a neurodegenerative disorder linked to repetitive brain injuries [45]. Each damaging event may lead to a specific clinical condition, which requires specific observation and care to prevent long-term neurological damage.

http://dx.doi.org/10.5772/intechopen.75745

Related head injuries can involve different motions of the event that ultimately impose a stretching force on neurons, commonly resulting in the dangerous formation of edema in the tissue, which increases tissue volume. Brain edema is influenced by complex molecular and cellular changes in blood–brain barrier (BBB) function, as well as cell volume regulation. These changes may also develop into pathological pathways. Edema resulting from the original sustained injury has a devastating impact on morbidity and mortality. These downstream effects of TBI increase intracranial pressure, impair cerebral perfusion and oxygenation, and contribute to additional ischemic injuries [46]. Therefore, these changes may also develop into pathological pathways. Other issues related to cerebral hypoperfusion range from loss of consciousness to devastating neuronal damage. The reason for these symptoms is the lack of high-energy phosphate compounds and high-energy metabolic demand caused by disruption of the continuous oxygen supply in the blood to the brain.

In general, there are three major types of traumatic brain edema. The first is *vasogenic* due to disruption of the BBB, which results in extracellular water accumulation. The second is *cytotoxic/cellular* due to sustained intracellular water collection. The third is called *osmotic* brain edema, which happens because of osmotic imbalances between blood and tissue. Rarely after TBI do we encounter a "hydrocephalic edema/interstitial" brain edema related to an obstruction of cerebrospinal fluid outflow [47]. Various detailed case studies have emerged that continue to raise the alarm and grab the attention of researchers to understand the effects of TBI. For many repeated types of injuries to the head, in certain individuals CTE has similarities to age-related neurodegenerative diseases [1]. Model systems such as rats [4, 6, 12, 13, 15, 17, 28, 29, 32, 33] and mice [5, 9, 11, 12, 18, 20, 25] have been employed to better understand the mechanisms of TBI.

Phosphorus is a very important element in the body and is responsible for approximately 1.1% of total body mass. In the body, almost all of the phosphorus is combined with oxygen, forming phosphate. Phosphate acts as a body's electrolytes, carrying an electric charge in body fluids such as blood. The majority of phosphate in the body (85%) comes from bone [48]. The rest is stored as high-energy phosphate or in its free form, where it acts as a substrate for adenosine triphosphate (ATP) production. Even though phosphate metabolism in trauma has not been well studied, there are some interesting reports on phosphate in TBI that involve hypophosphatemia. In 2010 Lindsey et al studied 25 patients with TBI and found out that these individuals had a lower serum phosphorus concentration than those without TBI, suggesting ongoing phosphate loss in the TBI patients [49].

To date, conventional computed tomography (CT) is the main technique for the evaluation of TBI for patients' diagnoses. However, CT and magnetic resonance imaging (MRI) still cannot be used to predict neurocognitive functional deficits at any stage of TBI, because they do not image the functional pathology for the neurocognitive outcome [50]. Therefore, other techniques such as ^{31}P-magnetic resonance imaging/spectroscopy (^{31}P-MRI/MRS) and positron emission tomography (PET) are used as alternatives to provide insight into the metabolic changes that arise from TBI and to reveal the damage that contributes to short- and long-term impairment. In this chapter, a review of several relevant contributions of neuroimaging towards an improved understanding of TBI is presented, using both PET and ^{31}P-MRI/MRS.

3. MRI techniques for TBI that involve phosphates

In terms of MRI for TBI, various techniques have been employed (**Figures 1** and **2**). For example, T_2-weighted MRI has been used [5]. Interestingly, in 1990 Heiss et al. used PET of [^{18}F]fluorodeoxyglucose (FDG)coupled with ^{31}P-MRS to diagnose tumors in the brain. The study suggested that both methods can examine different aspects of tumors in the brain and can be used as a tool for further classification of brain tumors or diseases related to the brain such as TBI [51]. A further study in 2002 by Greenman et al. used a method called three-dimensional rapid acquisition with relaxation enhancement (RARE) pulse sequence for direct measurement of phosphocreatine (PCr) images of the human myocardium. The aim of this study was to assess the metabolic state of myocardial tissue in several disease states and determine the efficacy of therapeutic mediation [52]. Then, in 2005, Greenman et al. published a work related to ^{31}P-MRS to evaluate the metatarsal head region of the foot in neuropathic diabetic patients. The study concluded that a very uniform net magnetization can be achieved and the use of double-tuned birdcage radiofrequency coils can improve the quality of MRI/MRS examinations [50]. A study in 2018 conducted by Chen et al. using in vivo ^{31}P-MRS magnetization transfer (MT) suggested that MRS provides a direct measure of neuronal activity at the metabolic level by investigating the change in cerebral ATP metabolic rates in healthy adults upon repeated stimulation [45]. ^{31}P-MRS has also proven effective in detecting a selective saturation sequence for ATP and phospholipids. Thus, the ^{31}P-MRS-MT technique at 3 T is a good candidate for neurological and neuropsychiatric disorders because of the noninvasive nature of NMR studies. Additionally, ^{31}P-MRS was reported and discussed in 2004 by Cernak et al. [6] The technique involving metals such as manganese is also applicable: manganese-enhanced MRI was used in a 2011 study by Tang et al. [29] Additionally, ex vivo diffusion tensor imaging was implemented in a study in 2012 by Jin and coworkers [5].

^{1}H, ^{31}P, and ^{13}C in vivo MRS are complementary techniques that allow noninvasive measurement of different aspects of brain metabolism that may contribute to the clinical management of patients with acute TBI [53]. ^{13}C-MRS measures the breakdown of intake of ^{13}C-labeled sugar (e.g., glucose) via glycolysis and the tricarboxylic acid cycle. Even though not many ^{13}C-MRS studies have been conducted, the development of in vivo hyperpolarized techniques shows a potential to detect TBI. On the other hand, ^{31}P-MRS allows measurement of high-energy phosphates (ATP and PCr) produced by oxidative phosphorylation and creatine kinase in mitochondria [54]. Changes in these metabolites have been noted in several patients and animal studies (further study might reveal the role of the high-energy phosphates). ^{1}H-MRS is the most commonly used MRS technique for studying brain metabolism following TBI. It has the potential to measure various metabolites: some are associated such as lactate, Glu and Gln, which can also be measured by ^{13}C-MRS. Creatine and N-acetylaspartic acid are associated with the ATP and PCr, which can also be measured with ^{31}P-MRS. Thus, the ratios of high-energy phosphates are thought to represent a balance in the brain. In addition, the chemical shift difference between inorganic phosphate and PCr enables calculation of intracellular pH. ^{13}C-MRS detects the ^{13}C isotope of carbon in brain metabolites [55].

http://dx.doi.org/10.5772/intechopen.75745

4. Molecules of importance

There are various small molecules used either as diagnostic agents or potential therapies in the context of phosphate TBI studies. We can also consider small molecule probes and those coupled with the use of pharmaceuticals (**Figures 3–6**).

5. PET imaging

PET is an important clinically used instrumental technique that requires an administration of artificial diagnostic agents (**Figure 1**). The artificial agents used involve one disintegrating atom such as the ^{18}F or ^{11}C isotope. The isotope is generated and then covalently attached (by a simple chemical reaction and protocol) to a small molecule prior to nuclear medical examination [13, 21].

PET imaging is well known for its sensitivity for small molecular changes (nanogram scale) compared to milligram or microgram for MRI or CT. PET also is able to provide important information on brain metabolism. As a result, PET imaging is used to measure a change in the glucose metabolism after TBI. The magnitude and duration have been correlated with worse behavioral and cognitive outcomes [56]. These results regarding cerebral glucose utilization were obtained using deoxyglucose (DG) labeled with ^{14}C and autoradiography [57]. DG was chosen because DG is phosphorylated but not further metabolized, becoming trapped in the cell with a slow clearance rate. For noninvasive imaging, a positron-emitting isotope such as ^{18}F can be incorporated within DG, resulting in the production of [^{18}F]FDG; this then accumulates in brain tissue in proportion to glucose uptake and the level of phosphorylation and is quantifiable using the technique of PET imaging [58].

For more information on nuclear chemistry and the mechanism of positron/electron capture as well as the preparative chemistry, please see other sources.

6. Phosphate species

There is a range of phosphate species used in biology. In some ways, the phosphates are central to the discussion, but in other ways they are peripheral to the thrusts of literature reports. The phosphates under discussion are shown in **Figure 3** and listed below.

7. Phosphorylation

Phosphorylation of proteins (serine, threonine, and tyrosine), for example, is an essential theme in biology. It is a constantly monitored and investigated process in biological systems, and continues as a vital aspect in the study of neurodegenerative disease research because it

relates to kinase and phosphatase activity (**Figure 3**). For example, tau protein has been central in Alzheimer's disease (AD) hypotheses for many years. Hyperphosphorylation is considered to be an important step in disease pathology [59].

Among many kinases proteins, mitogen-activated protein kinases (MAPKs), protein kinase B (also known as Akt), and glycogen synthase kinase (GSK) are the major kinases involved in cellular signaling, and as confirmed by the study from Joseph T. Neary, MAPKs, Akt, and GSK respond to trauma of the central nervous system (CNS). Therefore, it is very important to conduct further studies of these proteins to provide a better understanding of their role in the pathogenesis of many disorders, including traumatic injuries of the brain [58]. A study by Naoki Otani et al. showed that the extracellular signal-regulated kinase (ERK) pathway is triggered in lesions in regions of selective vulnerability after TBI and has a devastating effect on the hippocampus. The results show that pretreatment with U0126 (an ERK inhibitor) decreases neuronal cell loss after TBI [60]. Meanwhile, a study conducted by Noshita et al. also suggested that phosphorylation of Akt at serine-473 and DNA fragmentation after TBI in mice showed that phospho-Akt was decreased in the injured cortex 1 h after TBI and temporarily increased at 4 h in the perifocal damaged cortex. They concluded that the degree of Akt phosphorylation is dependent on the intensity of cellular damage after TBI [61].

Another study revealed that MAPKs are involved in pathophysiological TBI. Thus, regulating the MAPK pathway-mediated cerebral damage after acute injury could be a direction for the development of the novel therapeutic target in TBI [62–64]. Study of a simple chemical compound, sodium selenite, was performed. Sodium selenite was found to upregulate proteins that help to remove the phosphorylation group from its position on the amino acids in particular proteins. The specific enzyme is called PP2A/PR55 (protein phosphatase 2A regulatory subunit PR55). In a study from 2014 by Zhu et al., phosphorylation of various molecules was considered as a result of cerebral contusion (mouse model). The following molecules were studied: Akt, mTOR (mammalian target of rapamycin), and S6RP [35]. For example, the Thr308 and Ser473 sites of Akt are important phosphorylation sites for activating Akt. Thr308 becomes phosphorylated by PKD1 and other enzymes, including PDK2 phosphorylate Ser473. Activated Akt mediates several responses, including phosphorylating a range of intracellular proteins. mToR and S6RP are downstream targets of the PI3K/Akt pathway. Phosphorylation of a precursor stimulates activation of mTOR and S6RP [65–67]. Some phospholipids are ubiquitous and have been the subject of imaging regarding cell membrane dynamics.

8. Other phosphates

Various free, small, and organically bound phosphates are encountered in the phosphate imaging TBI literature:

- Pentose phosphate (see **Figure 3**)
- ATP and its dynamics [8]
- Reduced nicotinamide adenine dinucleotide phosphate (NADPH)

- *N*-acyl-phosphatidylethanolamines [20]
- Lysophosphatidylcholine [20]
- Ceramide phosphate [20]
- Bis(monoacylglycero)phosphate [20]
- Sphingosine-1-phosphate [20]
- Lysophosphatidylserine [20]
- *N*-acylethanolamine phospholipids

The result from Emily V. Mesev et al. proposes that the endogenous production of ceramide-1-phosphate (C1P) via ceramide kinase in brain tissue increases the basal activity of P-glycoprotein and contributes to general neuroprotection in healthy brains within the BBB. In cases of cellular injury or stress, it is possible that increases in C1P would act as a neuroprotector [68].

A study from Alice E. Pasvogel et al. showed that following TBI, membrane integrity of neurons and neuroglia is compromised resulting in elevated phospholipid levels in the cerebrospinal fluid. The pattern of change and the concentration of each of the phospholipids were different for those who died and those who survived following TBI. In conclusion, the study found the increase concentration of lysophosphatidylcholine in those who died. These findings give a preliminary proof of greater disruption of central nervous system membrane phospholipids in patients who died after TBI [69, 70].

9. Extracellular phosphates

In addition to the endogenous phosphate species that are produced in the biological system, there are also exogenous or xenobiological compounds that can be discussed. Chitosan combined with β-glycerophosphate disodium (β-GP) for use as a thermosensitive hydrogel was first reported by Chenite in 2000. This gel-forming biopolymer can be used for the development of therapeutic implants. Further study by Dong et al. from 2015 involved a hydrogel that consisted of derivatives of phosphate groups [10]. The result suggests that an injectable thermosensitive chitosan/gelatin/β-glycerol phosphate (C/G/GP) hydrogel could release the phenolic antioxidant ferulic acid (FA), which can inhibit the neurological oxidative stress and effectively protect the brain from further impairments. Another study from Ibrahim Jalloh et al. in 2015 also suggested that there was a shift in glucose metabolism from glycolysis to pentose phosphate pathways (PPPs) with decreasing brain tissue oxygen concentrations after TBI. This finding gives another perspective on the roles of PPPs and glycolysis after TBI, and whether they can be manipulated to enhance the potentially antioxidant role of PPPs and give better outcome to TBI patients [71].

In 2014, Brend L. Fiebich et al. suggested that prostaglandin E2 (PGE2), produced by the enzymatic activity of cyclooxygenases (COX) 1 and 2, is the common mediator for the inflammatory brain that leads to TBI. The group proposed a two-hit model for neuronal injury. First, an initial localized inflammation mediated by PGE2 was then followed by the release of ATP

by injured cells (second hit). In this study, it was concluded that by inhibiting the P2 receptor in the second hit using P2 receptor-based antiinflammatory drugs (PBAIDs) the activity of specific ectonucleotidases and release of excessive ATP could be increased and is another approach to counter neuroinflammation [72, 73].

10. MRI contrast agents

In TBI phosphate literature, MRI contrast agents have been previously described [31]. Structural information about the brain can be quantified using brain volume based on T_1-weighted MRI. Even though the most common contrast agents are based on gadolinium, new pharmaceuticals (for example, gadobenate benate dimeglumine (Gd-BOPTA)) have been developed with higher T_1 and T_2 relaxivity to improve signal intensity enhancement and thereby improve lesion visualization [74]. Garcia-Martin et al. used a phosphonated Gd^{3+}-based contrast agent to measure intravascular acidification in rat gliomas. To distinguish the differences in pH, [75, 76] the contrast agent used undergoes changes in T_1 relaxivity over a broad range from pH 6 to 8 [77]. This application is an example of an alternative for TBI symptom detection.

Another study using manganese-enhanced MRI (MEMRI), in which the manganese ion acts as an MRI contrast agent, was used to study rats subjected to a controlled cortical impact. The results suggest that MEMRI detected early indications of excitotoxic injury and BBB disruption that preceded vasogenic edema in the hyperacute phase and offer a novel contrast that complements conventional MRI in the study of TBI [78, 79]. In 2009 Chapon et al. revealed that MRI contrast agent can detect the inflammatory progression by radiolabeled peptide (IELLQAR) to target E-selectin, an important intercellular adhesion molecule involved in the leukocyte cycle [78].

11. Therapeutics tested

Small molecules are at the heart of medically treating people who have received TBI. (*R,S*)-2-Chloro-5-hydroxyphenylglycine (CHPG [5]) was studied. This compound has been studied by David J loane et al. in 2013 [80] and the result in mice model demonstrate that activation of mGluR5 using the selective agonist and CHPG, within 30 minutes after the moderate-level TBI significantly improved sensorimotor and cognitive function recovery and reduced TBI-induced lesion volumes in the mice model. Next, edaverone (**Figure 5**) was used [11]; it was found to be effective in the mouse model under study. The theme of concussion-induced depression is elucidated in this paper [11].

12. Proteins and enzymes

There are also related proteins in these studies. Perhaps the most central protein in a discussion of neurodegeneration is the beta amyloid protein (Aβ)—one of the hallmarks of AD. It

can be used as a baseline measurement. Studies by the Smith [81] and Sharp groups [82] showed that Aβ plaques may be found within TBI patients. The study also suggests that rapid Aβ plaque formation may result from the accumulation of amyloid precursor protein in damaged axons and a disturbed balance between Aβ genesis and catabolism during the process of TBI. In this study, the authors took an image of Aβ plaque burden in long-term survivors of TBI and made determinations to generate a correlation between traumatic axonal injury and Aβ concentration. By comparing the distribution of Aβ to AD, they found that Aβ-comprised plaque in the TBI survivors decreased in neocortical regions but increased in another brain region, the cerebellum. This then suggested that TBI may dispose one to an AD-like fate [25]. There are also phosphate-related reports involving studying the reduction of certain proteins after the onset of TBI. Such proteins include CREB and PSD95 [26]. Then there is carbamylated erythropoietin (EPO) [4]; EPO is a pleiotropic cytokine that identified its role in erythropoiesis (the process by which red blood cells are produced) [83]. EPO was recognized for its hematopoietic properties; however, many researchers around the globe were attracted by its function as a tissue protector. In 2004, a study from Leist et al. showed that the carbamylation of EPO formed a kind of nonhematopoietic derivative, cEpo. This reaction surprisingly eliminated its erythropoietic effects; however, it keeps its function in tissue protection [84]. These results led to another study conducted by Fiordaliso et al. from 2004, which suggested that the erythropoietic and tissue-protective effects of EPO were based on different receptors [85]. These discoveries have brought many researchers to design and synthesize EPO derivatives with tissue-protective effects only. To date, there are two major, developed, modified EPO molecules that have tissue-protective effects: cEpo and asialoerythroprotein (asialoEpo). Interestingly, the first modification of EPO through carbamylation was reported by Leist et al.; however, the method of producing cEpo was described in a patent by Warren Pharmaceuticals [86]. This newly reported research may shed new light on the development and application of cEpo, a prospective drug candidate for neuroprotection. There are studies that involve delayed mGluR5 activation and targeting of intermediate proteins [3]. One study found that activation of metabotropic glutamate receptor 5 (mGluR5) by CHPG decreases microglial activation and release of associated proinflammatory factors in vitro, which is mediated, in part, through inhibition of reduced NADPH oxidase. These results suggested that treatment with CHPG may significantly limit lesion progression in TBI through mGluR5 receptors [87].

13. Conclusions and future outlook

There are various ways that the wide variety of phosphates that exist in biology are involved in health and disease; ions such as phosphates can be exploited in many prospective ways in the future and in particular they could be imaged in new ways. This review concerned phosphates and TBI reports in which the discussion or study involved molecular imaging. The reports were clinical and involved laboratory studies. Animal models were often used. A great deal of biochemistry was described; often, enzyme activities were monitored and these trends were published.

This fresh review was intended to help medicinal chemists make new connections. *The major goals are intended to help achieve future innovation of potential treatment of TBI with chemical or biological agents. Administration within the "golden hour" for the best efficacy is an essential point to make.* In terms of imaging there are new MRI techniques and experiments that are available as well. Some of the most important instrument manufacturers such as GE Healthcare (Milwaukee, WI), Bruker, Hitachi Medical Corporation, Phillips, and Toshiba Medical Corporation provide the current hardware for the task at hand [88–92]. However, biochemistry can allow for additional innovative imaging to be undertaken. Below are a few detailed aspects for future study with regard to TBI and phosphate research.

13.1. Future

More commonly, research in the future will prominently feature the effects on phosphate metabolism. With phosphate metabolism still in its infancy [93], a fuller treatment would involve a great deal of related research. Therefore, we have described some related papers that involve important points about phosphates.

- Much research effort involves the status of enzyme activity. The importance of accurately carrying out immunohistochemistry involving phosphorylated proteins can be underscored [16, 47]. How well Western blots and other related assays are prepared and conducted by laboratory personnel and how they can be best carried out and executed are extremely important for the field.
- The theme of subcellular redistribution of phosphates can be made more pronounced [16]. Novel chemical probes that can "chase" the constituents between different cellular compartments can be designed and studied.
- The importance of the maintenance of vasculature and smooth muscle cells that help constitute the microvessels within neurological tissue can be further studied. How these structures are effected by TBI in, e.g., mouse models can be further determined [16].
- Overabundant Reactive Oxygen Species (ROS) concentration driven by Fenton reaction has major role in the transformation of many highly radical species such as ROS/RNS. These highly reactive species, can lead to many disturbances such as TBI. See references herein and elsewhere for an introduction to ROS and their analysis. MRI is a very common theme in research [1, 15, 28, 29, 31], as well as the closely related instrumental technique of NMR spectroscopy.
- How phosphates are interrelated (via brain injury) with the range of ROS is an important quest in basic science.
- More research about phosphates in *gliosis* needs to be researched. How can gliosis best be imaged and can it relate to the homeostasis of phosphates?
- What is the range of factors that delays mGluR5 activation and how do phosphates or phosphonates become involved?
- How can researchers parse between *secondary* and *primary* pathology at the chemical level regarding both experimental and clinical research of TBI phosphate activity?

http://dx.doi.org/10.5772/intechopen.75745

- What divergent effects might arise from prior organophosphate/organophosphonate pesticide exposure (a history of exposure) in which phosphonates are located where phosphorylation usually takes place? How does this effect hinder or perhaps help in etiology? How can medicine take advantage of this artificial preloading?

Abbreviations

AD	Alzheimer's disease
ADP	adenosine diphosphate.
ATP	adenosine triphosphate
Akt	protein kinase B
APOE	apolipoprotein
BBB	blood brain barrier
C1P	ceramide-1-phosphate
CBF	cerebral blood flow.
COX	cyclooxygenases
CREB	cAMP response element-binding protein
CTE	chronic traumatic encephalopathy
Gd-BOPTA	gadolinium benate dimeglumine
Gln	glutamine
Glu	glutamic acid
HR-MS	high resolution–mass spectroscopy.
KCl	potassium chloride.
KH_2PO_4	monopotassium phosphate.
MEMRI	manganese-enhanced MRI
mGluR5	metabotropic glutamate receptor 5
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
mTOR	mammalian target of rapamycin
NAA	*N*-acetylaspartic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NaCl	sodium chloride.
Na_2HPO_4	sodium hydrogen phosphate.

NMR	nuclear mass resonance
NOX2	nicotinamide adenine dinucleotide phosphate oxidase.
PGE2	prostaglandin E2
PBAID	P2 receptor-based antiinflammatory drugs
PBS	phosphate buffered saline.
PET	positron emission tomography
PP2A/PR55	protein phosphatase 2A regulatory subunit PR55
PSD95	postsynaptic density protein 95
TBI	traumatic brain injury
Tg mice	transgenic mice.
TP	triphosphate
S6RP	phosphorylation of S6 ribosomal protein

Author details

Noam Naphatali Tal[1], Tesla Yudhistira[2,4], Woo Hyun Lee[2], Youngsam Kim[2,3] and David G. Churchill[2,3,5]*

*Address all correspondence to: dchurchill@kaist.ac.kr

1 Independent Scientist and Former student, Department of Medical Device Technology, Maltash College, Tel Aviv, Israel

2 Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

3 Center for Catalytic Hydrocarbon Functionalizations, Institute for Basic Science (IBS), Daejeon, Republic of Korea

4 Lembaga Pengelola Dana Pendidikan (LPDP), Indonesia Endowment Fund for Education, Kementrian Keuangan Republik Indonesia, Jakarta, Indonesia

5 Schulich Faculty of Chemistry at Technion, Israel Institute of Technology, Haifa, Israel

References

[1] Nisenbaum EJ, Novikov DS, Lui YW. The presence and role of iron in mild traumatic brain injury: an imaging perspective. Journal of Neurotrauma. 2014;**31**(4):301

[2] Antonenko YN, Denisov SS, Silachev DN, Khailova LS, Jankauskas SS, Rokitskaya TI, Danilina TI, Kotova EA, Korshunova GA, Plotnikov EY, et al. A long-linker conjugate of fluorescein and triphenylphosphonium as mitochondria-targeted uncoupler and fluorescent neuro- and nephroprotector. Biochimica et Biophysica Acta - General Subjects. 2016;**1860**(11):2463

[3] Bargagna-Mohan P, Paranthan RR, Hamza A, Dimova N, Trucchi B, Srinivasan C, Elliott GI, Zhan CG, Lau DL, Zhu HY, et al. Withaferin A targets intermediate filaments glial fibrillary acidic protein and vimentin in a model of retinal gliosis. Journal of Biological Chemistry. 2010;**285**(10):7657

[4] Bouzat P, Francony G, Thomas S, Valable S, Mauconduit F, Fevre MC, Barbier EL, Bernaudin M, Lahrech H, Payen JF. Reduced brain edema and functional deficits after treatment of diffuse traumatic brain injury by carbamylated erythropoietin derivative. Critical Care Medicine. 2011;**39**(9):2099

[5] Jin H, Wu G, Hu S, Hua Y, Keep RF, Wu J, Xi G. T2 and T2* magnetic resonance imaging sequences predict brain injury after intracerebral hemorrhage in rats. Acta Neurochirurgica Supplement. 2013;**118**:151

[6] Cernak I, Vink R, Zapple DN, Cruz MI, Ahmed F, Chang T, Fricke ST, Faden AI. The pathobiology of moderate diffuse traumatic brain injury as identified using a new experimental model of injury in rats. Neurobiology of Disease. 2004;**17**(1):29

[7] Chantong B, Kratschmar DV, Lister A, Odermatt A. Inhibition of metabotropic glutamate receptor 5 induces cellular stress through pertussis toxin-sensitive Gi-proteins in murine BV-2 microglia cells. Journal of Neuroinflammation. 2014;**11**:16

[8] Connolly NMC, Prehn JHM. The metabolic response to excitotoxicity—lessons from single-cell imaging. Journal of Bioenergetics and Biomembranes. 2015;**47**(1–2):75

[9] DellaValle B. GLP-1 receptor activation improves neurological outcome after murine brain trauma (vol 1, pg 1, 2014). Annals of Clinical and Translational Neurology. 2016;**3**(8):664

[10] Dong GC, Kuan CY, Subramaniam S, Zhao JY, Sivasubramaniam S, Chang HY, Lin FH. A potent inhibition of oxidative stress induced gene expression in neural cells by sustained ferulic acid release from chitosan based hydrogel. Materials Science and Engineering C: Materials for Biological Applications. 2015;**49**:691

[11] Higashi Y, Hoshijima M, Yawata T, Nobumoto A, Tsuda M, Shimizu T, Saito M, Ueba T. Suppression of oxidative stress and 5-lipoxygenase activation by edaravone improves depressive-like behavior after concussion. Journal of Neurotrauma. 2014;**31**(20):1689

[12] Hill JL, Kobori N, Zhao J, Rozas NS, Hylin MJ, Moore AN, Dash PK. Traumatic brain injury decreases AMP-activated protein kinase activity and pharmacological enhancement of its activity improves cognitive outcome. Journal of Neurochemistry. 2016;**139**(1):106

[13] Hwang H, Jeong HS, Oh PS, Na KS, Kwon J, Kim J, Lim S, Sohn MH, Jeong HJ. Improving cerebral blood flow through liposomal delivery of angiogenic peptides: potential of

F-18-FDG PET imaging in ischemic stroke treatment. The Journal of Nuclear Medicine. 2015;**56**(7):1106

[14] Jin Y, Sui HJ, Dong Y, Ding Q, Qu WH, Yu SX, Jin YX. Atorvastatin enhances neurite outgrowth in cortical neurons in vitro via up-regulating the Akt/mTOR and Akt/GSK-3 beta signaling pathways. Acta Pharmacologica Sinica. 2012;**33**(7):861

[15] Karki K, Knight RA, Han YX, Yang DM, Zhang JF, Ledbetter KA, Chopp M, Seyfried DM. Simvastatin and atorvastatin improve neurological outcome after experimental intracerebral hemorrhage. Stroke. 2009;**40**(10):3384

[16] Kreipke CW, Morgan RL, Petrov T, Rafols JA. Subcellular redistribution of calponin underlies sustained vascular contractility following traumatic brain injury. Neurological Research. 2007;**29**(6):604

[17] 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. Journal of Neurotrauma. 1999;**16**(3):213

[18] Kumar A, Alvarez-Croda DM, Stoica BA, Faden AI, Loane DJ. Microglial/macrophage polarization dynamics following traumatic brain injury. Journal of Neurotrauma. 2016;**33**(19):1732

[19] Mannix R, Meehan WP, Mandeville J, Grant PE, Gray T, Berglass J, Zhang J, Bryant J, Rezaie S, Chung JY, et al. Clinical correlates in an experimental model of repetitive mild brain injury. Annals of Neurology. 2013;**74**(1):65

[20] Nielsen MMB, Lambertsen KL, Clausen BH, Meyer M, Bhandari DR, Larsen ST, Poulsen SS, Spengler B, Janfelt C, Hansen HS. Mass spectrometry imaging of biomarker lipids for phagocytosis and signalling during focal cerebral ischaemia. Scientific Reports. 2016;**6**:14

[21] Nortje J, Coles JP, Timofeev I, Fryer TD, Aigbirhio FI, Smielewski P, Outtrim JG, Chatfield DA, Pickard JD, Hutchinson PJ, et al. Effect of hyperoxia on regional oxygenation and metabolism after severe traumatic brain injury: preliminary findings. Critical Care Medicine. 2008;**36**(1):273

[22] Papisov MI, Belov VV, Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. Molecular Pharmaceutics. 2013;**10**(5):1522

[23] Park J, Zhang J, Qiu JH, Zhu XX, Degterev A, Lo EH, Whalen MJ. Combination therapy targeting Akt and mammalian target of rapamycin improves functional outcome after controlled cortical impact in mice. Journal of Cerebral Blood Flow & Metabolism. 2012;**32**(2):330

[24] Patrick MM, Grillot JM, Derden ZM, Paul DW. Long-term drifts in sensitivity caused by biofouling of an amperometric oxygen sensor. Electroanalysis. 2017;**29**(4):998

[25] Schwetye KE, Cirrito JR, Esparza TJ, Mac Donald CL, Holtzman DM, Brody DL. Traumatic brain injury reduces soluble extracellular amyloid-beta in mice: a methodologically novel combined microdialysis-controlled cortical impact study. Neurobiology of Disease. 2010;**40**(3):555

[26] Sen T, Gupta R, Kaiser H, Sen N. Activation of PERK elicits memory impairment through inactivation of CREB and downregulation of PSD95 after traumatic brain injury. The Journal of Neuroscience. 2017;**37**(24):5900

[27] Starkov AA, Polster BM, Fiskum G. Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax. Journal of Neurochemistry. 2002;**83**(1):220

[28] Tan XL, Wright DK, Liu SJ, Hovens C, O'Brien TJ, Shultz SR. Sodium selenate, a protein phosphatase 2A activator, mitigates hyperphosphorylated tau and improves repeated mild traumatic brain injury outcomes. Neuropharmacology. 2016;**108**:382

[29] Tang HL, Sun HP, Wu X, Sha HY, Feng XY, Zhu JH. Detection of neural stem cells function in rats with traumatic brain injury by manganese-enhanced magnetic resonance imaging. Chinese Medical Journal. 2011;**124**(12):1848

[30] Valable S, Francony G, Bouzat P, Fevre MC, Mahious N, Bouet V, Farion R, Barbier E, Lahrech H, Remy C, et al. The impact of erythropoietin on short-term changes in phosphorylation of brain protein kinases in a rat model of traumatic brain injury. Journal of Cerebral Blood Flow & Metabolism. 2010;**30**(2):361

[31] Van Horn JD, Bhattrai A, Irimia A. Multimodal imaging of neurometabolic pathology due to traumatic brain injury. Trends in Neurosciences. 2017;**40**(1):39

[32] Wu JG, Li H, Wang DS, Xu DS, Wang W. Intravenous adipose-derived stem cells transplantation ameliorates memory impairment in moderate traumatic brain injury rats via the phosphorylation of extracellular signal-regulated kinase 1/2. International Journal of Clinical and Experimental Medicine. 2016;**9**(7):12649

[33] Yu TG, Feng Y, Feng XY, Dai JZ, Qian HJ, Huang Z. Prognostic factor from MR spectroscopy in rat with astrocytic tumour during radiation therapy. The British Journal of Radiology. 2015;**88**(1045):10

[34] Zemlan FP, Rosenberg WS, Luebbe PA, Campbell TA, Dean GE, Weiner NE, Cohen JA, Rudick RA, Woo D. Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. Journal of Neurochemistry. 1999;**72**(2):741

[35] Zhu XX, Park J, Golinski J, Qiu JH, Khuman J, Lee CCH, Lo EH, Degterev A, Whalen MJ. Role of Akt and mammalian target of rapamycin in functional outcome after concussive brain injury in mice. Journal of Cerebral Blood Flow & Metabolism. 2014;**34**(9):1531

[36] Liu B, Wang H, Yang D, Tan R, Zhao RR, Rui X, Zhang JZ, Zhang JF, Ying Z. A cyanine-based colorimetric and fluorescent probe for highly selective sensing and bioimaging of phosphate ions. Dyes and Pigments. 2016;**133**:127

[37] Resa S, Orte A, Miguel D, Paredes JM, Muñoz VP, Salto R, Giron MD, Rama MJR, Cuerva JM, Pez JMA, Crovetto L. New dual fluorescent probe for simultaneous biothiol and phosphate bioimaging. Chemistry: A European Journal. 2015;**21**:14772

[38] Paredes JM, Giron MD, Rama MJR, Orte A, Crovetto L, Talavera EM, Salto R, Pez JMA. Real-time phosphate sensing in living cells using fluorescence lifetime imaging microscopy (FLIM). Journal of Physical Chemistry B. 2013;**117**(27):8143

[39] Banerjee S, Versaw WK, Garcia LR. Imaging cellular inorganic phosphate in Caenorhabditis elegans using a genetically encoded FRET-based biosensor. PLoS One. 2015;**10**(10):1

[40] Janich K, Nguyen HS, Patel M, Shabani S, Montoure A, Doan N. Management of adult traumatic brain injury: a review. Journal of Trauma and Treatment. 2016;**5**:1

[41] Weaver CS, Sloan BK, Brizendine EJ, Bock H. An analysis of maximum vehicle g forces and brain injury in motorsports crashes. Medicine & Science in Sports & Exercise. 2006;**38**:246

[42] Nakagawa A, Fujimura M, Kato K, Okuyama H, Hashimoto T, Takayama K, Tominaga T. Shock wave-induced brain injury in rat: novel traumatic brain injury animal model. Acta Neurochirurgica Supplement. 2008;**102**:421

[43] Nakagawa A, Manley GT, Gean AD, Ohtani K, Armonda R, Tsukamoto A, Yamamoto H, Takayama K, Tominaga T. Mechanisms of primary blast-induced traumatic brain injury: insights from shock-wave research. Journal of Neurotrauma. 2011;**28**:1101

[44] Wang H, Zhang YP, Cai J, Shields LB, Tuchek CA, Shi R, Li J, Shields CB, Xu XM. Compact blast-induced traumatic brain injury model in mice. Journal of Neuropathology & Experimental Neurology. 2016;**75**:183

[45] Chen C, Stephenson MC, Peters A, Morris PG, Francis ST, Gowland PA. 31P magnetization transfer magnetic resonance spectroscopy: assessing the activation induced change in cerebral ATP metabolic rates at 3 T. Magnetic Resonance in Medicine. 2018;**79**:22

[46] Unterberg AW, Stover J, Kress B, Kiening KL. Edema and brain trauma. Neuroscience. 2004;**129**:1021

[47] Arur S, Schedl T. Generation and purification of highly-specific antibodies for detecting post-translationally modified proteins in vivo. Nature Protocols. 2014;**9**(2):375

[48] Penido MGMG, Alon US. Phosphate homeostasis and its role in bone health. Pediatric Nephrology (Berlin, Germany). 2012;**27**:2039-2048

[49] Lindsey KA, Brown RO, Maish GO 3rd, et al. Influence of traumatic brain injury on potassium and phosphorus homeostasis in critically ill multiple trauma patients. Nutrition (Burbank, Los Angeles County, Calif). 2010;**26**:784-790

[50] Greenman RL, Rakow-Penner R. Evaluation of the RF field uniformity of a double-tuned 31P/1H birdcage RF coil for spin-echo MRI/MRS of the diabetic foot. Journal of Magnetic Resonance Imaging. 2005;**22**:427

[51] Heiss WD, Heindel W, Herholz K, Rudolf J, Bunke J, Jeske J, Friedmann G. Positron emission tomography of fluorine-18-deoxyglucose and image-guided phosphorus 31 magnetic resonance spectroscopy in brain tumors. Journal of Nuclear Medicine. 1990;**31**:302

[52] Greenman RL, Axel L, Ferrari VA, Lenkinski RE. Fast imaging of phosphocreatine in the normal human myocardium using a three-dimensional RARE pulse sequence at 4 Tesla. Journal of Magnetic Resonance Imaging. 2002;**15**:467

[53] Mountford CE, Stanwell P, Lin A, Ramadan S, Ross B. Neurospectroscopy: The past, present and future. Chemical Reviews. 2010;**110**(5):3060

[54] Cernak I, Vink R, Zapple DN, Cruz MI, Ahmed F, Chang T, Fricke ST, Faden AI. The pathobiology of moderate diffuse traumatic brain injury as identified using a new experimental model of injury in rats. Neurobiology of Disease. 2004;**17**:29

[55] Stovell MG, Yan JL, Sleigh A, Mada MO, Carpenter TA, Hutchinson PJA, Carpenter KLH. Assessing metabolism and injury in acute human traumatic brain injury with magnetic resonance spectroscopy: current and future applications. Frontiers in Neurology. 2017;**8**:426

[56] Giza CC, Hovda DA. The neurometabolic cascade of concussion. Journal of Athletic Training. 2001;**36**:228

[57] Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD. The [14C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. Journal of Neurochemistry. 1977;**28**:897-916

[58] Neary JT. Protein kinase signaling cascades in CNS trauma. IUBMB Life. 2005;**57**:711

[59] Franke H, Verkhratsky A, Burnstock G, Illes P. Pathophysiology of astroglial purinergic signalling. Purinergic Signalling. 2012;**3**:629

[60] Otani N, Nawashiro H, Fukui S, Ooigawa H, Ohsumi A, Toyooka T, Shima K. Role of the activated extracellular signal-regulated kinase pathway on histological and behavioral outcome after traumatic brain injury in rats. Journal of Clinical Neuroscience. 2007;**14**:42

[61] Noshita N, Lewén A, Sugawara T, Chan PH. Akt phosphorylation and neuronal survival after traumatic brain injury in mice. Neurobiology of Disease. 2002;**9**:294

[62] Mori T, Wang X, Jung JC, Sumii T, Singhal AB, Fini ME, Dixon E, Alessandrini A, Lo EH. Mitogen-activated protein kinase inhibition in traumatic brain injury: in vitro and in vivo effects. Journal of Cerebral Blood Flow & Metabolism. 2002;**22**:444

[63] Walker CL, Liu NK, Xu XM. PTEN/PI3K and MAPK signaling in protection and pathology following CNS injuries. Frontiers in Biology (Beijing). 2013;**8**:1

[64] Otani N, Nawashiro H, Fukui S, Nomura N, Yano A, Miyazawa T, Shima K. Differential activation of mitogen-activated protein kinase pathways after traumatic brain injury in the rat hippocampus. Journal of Cerebral Blood Flow & Metabolism. 2002;**22**:327

[65] Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;**129**: 1261

[66] Nicholson KM, Anderson NG. The protein kinase B/Akt signaling pathway in human malignancy. Cellular Signalling. 2002;**14**:381

[67] Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes & Development. 2004;**18**:1926

[68] Mesev EV, Miller DS, Cannon RE. Ceramide-1-phosphate increases P-glycoprotein transport activity at the blood-brain barrier via prostaglandin E2 signaling. Molecular Pharmacology. 2017;**91**:373

[69] Pasvogel AE, Miketova P, Moore IM. Differences in CSF phospholipid concentration by traumatic brain injury outcome. Biological Research for Nursing. 2010;**11**:325

[70] Pasvogel A, Miketova P, Moore IM. Cerebrospinal fluid phospholipid changes following traumatic brain injury. Biological Research for Nursing. 2008;**10**:113

[71] Jalloh I, Carpenter KLH, Grice P, Howe DJ, Mason A, Gallagher CN, Helmy A, Murphy MP, Menon DK, Carpenter TA, Pickard JD, Hutchinson PJ. Glycolysis and the pentose phosphate pathway after human traumatic brain injury: microdialysis studies using 1,2-13C2 glucose. Journal of Cerebral Blood Flow & Metabolism. 2015;**35**:111

[72] Fiebich BL, Akter S, Akundi RS. The two-hit hypothesis for neuroinflammation: role of exogenous ATP in modulating inflammation in the brain. Frontiers in Cellular Neuroscience. 2014;**8**:1

[73] Franke H, Illes P. Nucleotide signaling in astrogliosis. Neuroscience Letters. 2014;**565**:14

[74] Maravilla KR. Gadobenate dimeglumine-enhanced MR imaging of patients with CNS diseases. European Radiology. 2006;**16**:8

[75] Chapon C, Franconi F, Lacoeuille F, Hindré F, Saulnier P, Benoit JP, Le Jeune JJ, Lemaire L. Imaging E-selectin expression following traumatic brain injury in the rat using a targeted USPIO contrast agent. Magnetic Resonance Materials in Physics, Biology and Medicine. 2009;**22**:167

[76] Garcia-Martin ML, Martinez GV, Raghunand N, Sherry AD, Zhang S, Gillies RJ. High resolution pH(e) imaging of rat glioma using pH-dependent relaxivity. Magnetic Resonance in Medicine. 2006;**55**:309

[77] Raghunand N, Zhang S, Sherry AD, Gillies RJ. In vivo magnetic resonance imaging of tissue pH using a novel pH-sensitive contrast agent, GdDOTA-4AmP. Academic Radiology. 2002;**9**:481

[78] Watts LT, Shen Q, Deng S, Chemello J, Duong TQ. Manganese-enhanced magnetic resonance imaging of traumatic brain injury. Journal of Neurotrauma. 2015;**32**:1001

[79] Jasanoff A. MRI contrast agents for functional molecular imaging of brain activity. Current Opinion in Neurobiology. 2007;**17**:593

[80] Loane DJ, Stoica BA, Byrnes KR, Jeong W, Faden AI. Activation of mglur5 and inhibition of nadph oxidase improves functional recovery after traumatic brain injury. Journal of Neurotrauma. 2013;**30**(5):403-412. http://doi.org/10.1089/neu.2012.2589

[81] Johnson VE, Stewart W, Smith DH. Traumatic brain injury and amyloid-β pathology: a link to Alzheimer's disease? Nature Reviews Neuroscience. 2010;**11**(5):361

[82] Scott G, Ramlackhansingh AF, Edison P, Hellyer P, Cole J, Veronese M, Leech R, Greenwood RJ, Turkheimer FE, Gentleman SM, Heckemann RA, Matthews PM, Brooks DJ, Sharp DJ. Amyloid pathology and axonal injury after brain trauma. Neurology. 2016;**86**(9):821

[83] Chen J, Yang Z, Zhang X. Carbamylated erythropoietin: a prospective drug candidate for neuroprotection. Biochemistry Insights. 2015;**8**:25

[84] Torup L. Neuroprotection with or without erythropoiesis; sometimes less is more. British Journal of Pharmacology. 2007;**151**(8):1141

[85] Brines M, Grasso G, Fiordaliso F. Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(41):14907

[86] Warren Pharmaceuticals. Novel carbamylated EPO and method for its production. WO2006/014466; 2006

[87] Byrnes KR, Loane DJ, Stoica BA, Zhang J, Faden AI. Delayed mGluR5 activation limits neuroinflammation and neurodegeneration after traumatic brain injury. Journal of Neuroinflammation. 2012;**9**:43

[88] Fishman RA. Brain Edema. The New England Journal of Medicine 1975;**293**:706-711. http://www.nejm.org/doi/full/10.1056/NEJM197510022931407

[89] Available from: https://www.bruker.com/products/mr/preclinical-mri.html

[90] Available from: http://www3.gehealthcare.com/en/products/categories/magnetic_resonance_imaging

[91] Available from: https://www.usa.philips.com/healthcare/solutions/magnetic-resonance

[92] Available from: http://www.hitachimed.com/products/mri

[93] Horsman GP, Zechel DL. Phosphonate biochemistry. Chemical Reviews. 2017;**117**(8):5704

IntechOpen

IntechOpen