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Exposure to lead in the pre- and neonatal periods may result in brain inflammation

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ABSTRACT

One of the proinflammatory agents in the human body is lead (Pb), which can enter the blood through the skin, respiratory tract and digestive tract, causing poisoning. Its most significant target is the central nervous system (CNS). Although studies on Pb neurotoxicity have been conducted for many years, the proinflammatory effect of Pb on the brain is rarely reported in contrast to other human tissues and organs. Lead neurotoxicity has been defined as a significant paediatric health problem as the foetal stage is a very susceptible period for Pb exposure at whole blood levels below 10 μg/dL (Pb neurotoxicity threshold in children). However, the mechanisms of the neurotoxic action of Pb in causing brain defects remain unclear. In this review we discuss the role of the blood-brain barrier in the neurotoxicity of Pb, and the role of cytokines as inflammatory mediators (specially interleukin-1 and interleukin-6, nuclear transcription factor κB, cyclooxygenase-1 and cyclooxygenase-2, prostaglandin E_2, transforming growth factor β. We also discuss the influence of pre- and neonatal exposure to Pb on inflammatory reactions in the brain.

Keywords: lead; inflammation; cytokines; cyclooxygenases (COX); prostaglandin E_2 (PGE_2); transforming growth factor beta (TGF-β).

INTRODUCTION

Inflammation is a non-specific response of the innate immune system, ensuring a rapid defence by the host against infection – hence it can be considered a protective response. However, the cytotoxic mechanisms of inflammation which combat infectious microorganisms and infected cells can also cause damage to healthy tissues. Inflammation can also occur in the absence of infection, e.g. during injury, hypoxia/ischemia (a so-called sterile inflammation [1]), and is increasingly recognised as one of the causes of some of the most common diseases [2].

One such proinflammatory agent in the human body is lead (Pb), which can enter the blood through the skin, respiratory tract and digestive tract, causing poisoning. Its most significant target is the central nervous system (CNS) [3]. Although studies on Pb neurotoxicity have been conducted for many years [4, 5, 6, 7, 8], the proinflammatory effect of Pb on the brain is rarely reported, in contrast to other human tissues and organs [3].

Lead neurotoxicity has been defined as a significant paediatric health problem [3]. The fetal stage is a very susceptible period for Pb exposure at whole blood levels below 10 μg/dL, the determined Pb neurotoxicity threshold in children [9]. However, the mechanisms of the neurotoxic action of Pb in causing brain defects remain unclear [10].

THE ROLE OF THE BLOOD-BRAIN BARRIER IN THE NEUROTOXICITY OF PB

The developing brain is a site of intense proliferation, differentiation and synaptogenesis. At this stage it is also very sensitive to environmental hazards. One such hazard, Pb exposure, can have serious consequences for brain development, with the hippocampus being the most sensitive to Pb (although the exact mechanism is yet unclear) [3].

An increasing body of evidence suggests that the blood-brain barrier plays a key role in the development or progression of CNS diseases [11, 12, 13]. Disruption of the structure and function of the blood-brain barrier associated with neuronal dysfunction may probably contribute to neurodegenerative diseases and other neuropathological events, such as multiple sclerosis (MS), stroke, ischemia, hemorrhage and infarction [14, 15]. The mechanisms responsible for disruption of the blood-brain barrier and the resulting consequences are complex. However, inflammation is seen as one of the main factors – for example, disorders of the blood-brain barrier causing neonatal white matter injury (NWMI) have been attributed to an unregulated inflammatory response [16]. Neuroinflammatory processes act as a double-edged sword because they are at the same time beneficial, supporting regenerative events, and harmful, strengthening local destructive pathways [17].

Microglial cells and infiltrated macrophages contain inflammasome complexes [18]. In severe brain injury, microglial cells react quickly [19], playing a decisive role in controlling and spreading secondary damage in spinal cord injuries [20], and in releasing proinflammatory cytokines and chemokines [19]. The 2 main subtypes of microglial cells (M1 and M2) perform different functions. M1 cells resemble activated microglia and produce a large number of proinflammatory cytokines [17]. In contrast, alternatively activated M2 cells are rather protective and promote tissue remodelling, wound healing and angiogenesis [21].

To date, the functional roles of proinflammatory factors induced by Pb have not been fully defined. On one hand, proinflammatory events interfere with the functioning of residual neural circuits, thus preventing remyelination and regeneration of axons, while on the other hand, proinflammatory factors may also participate in wound healing following CNS damage [3]. Reactive astrocytes are used to regulate molecular and ionic content in extracellular space at the site of an injury and induce neurotrophic factors [3].

Given the critical importance of astrocytes for many developmental events [22], toxic damage to astrocytes may impair CNS function. Glial fibrillary acidic protein (GFAP) is the main protein of glial intermediate filaments in differentiated astrocytes [23]. One of the most commonly recognized features of astrocytes in the mature brain is reactive gliosis, a reaction to CNS damage characterized by the presence of large amounts of reactive astrocytes, distinguished from normal astrocytes by their larger size and increased amount of GFAP [24]. For example, reactive gliosis is a characteristic feature of human NWMI [25, 26, 27] and may have either protective or harmful effects [28, 29].

Reactive astrocytes have been divided into 2 subtypes – A1 and A2 – based on different molecular markers. Reactive astrocytes expressing A1 markers are present in many neurodegenerative states in adult humans and are believed to cause neurotoxic effects. However, in transcriptional evaluations of reactive astrocyte substitutes in mouse models, the induction of cyclooxygenase-2 (COX-2) was associated rather with the A2 phenotype [30, 31]. The role of A2 astrocytes in "neuroinflammatory" lesions is unclear, and human neuropathic disorders associated with A2 have not yet been documented.

THE ROLE OF CYTOKINES AS INFLAMMATORY MEDIATORS

Interleukin-1

Most importantly, soluble sterile proinflammatory cytokines are members of the interleukin-1 (IL-1) family [2]. There are 11 members in the IL-1 family, of which the best known are the proinflammatory cytokines IL-1α and IL-1β, and the IL-1 receptor anti-inflammatory antagonist IL-1RA [1].

Interleukin-1α and IL-1β bind to IL-1 receptor type 1 (IL-1R1), triggering a cascade of signalling events that cause additional expression of proinflammatory cytokines [32], an increase in circulating leukocytes [33], greater vascular activation [34], increased neuronal excitability [35] and the activation of neurotoxicity via glial cells [36]. The interleukin-1 receptor antagonist (IL-1RA) blocks these effects by binding to IL-1R1 [1]. Interleukin-1β is produced as pro-IL-1β, an inactive precursor which remains bound to the cell after expression. The processing of pro-IL-1β into a mature active form is regulated by caspase-1 protease [37].

Data published by Gustin et al. [38] show that microglia are the main cell type in the brain responsible for the secretion of IL-1β. de Rivero Vaccari et al. [39] have suggested that rather than the microglia, it is the neurons that are the significant source of IL-1β in the CNS. This discrepancy may be caused by the fact that the microglia and neurons react at different times following injury. Neurons can be a very early line of defence following an injury as they are the main type of cells sensitive to oxygen and adenosine triphosphate (ATP) depletion. Therefore, neurons can send early signals to neighbouring immune cells, such as astroglia and microglia, which then take the next steps in local defensive action [40]. Holmin and Mathiesen describe the induction of apoptosis and inflammatory response in rats following an experimental injection of IL-1β into the midbrain after a serious brain injury [41]. Overproduction of IL-1β together with excessive inflammatory reactions may result in breaking the blood-brain barrier and producing neurotoxic molecules, which are involved in secondary cell death in severe brain injury [19] and spinal cord injury [42]. Clausen et al. [43] noted that the use of antibodies neutralising IL-1β after severe brain injury reduces the extent of damage and prevents histopathological damage.

Interleukin-1α is also produced as a precursor (pro-IL-1α), and although it shows some activity after binding to IL-1R1 [44], processing into a mature form increases its activity [45, 46]. Processing of pro-IL-1α seems to depend on calpain-like protease, although inflammatory complexes may play an indirect role in its activation [47]. Interleukin-1α can also be released by programmed necrosis or necroptosis [48] (a type of cell death that is increasingly recognized as the cause of inflammatory disease [49]). Although both IL-1α and IL-1β are common in many inflammatory states, recent evidence suggests that IL-1α is the dominant ligand that mediates early inflammation after the occurrence of encephalitis, e.g. in response to brain ischemia [50, 51].

There is extensive evidence that IL-1 is a key mediator of disorders in acute brain damage, especially in strokes, such as in the study by Boutin et al., showing the pathological involvement of IL-1 in neuropathic conditions such as stroke [52], which is partly due to neutrophil neurotoxicity [33].

To confirm this thesis, Giles et al. conducted an experiment in which inflammation was stimulated by a bacterial endotoxin (LPS) in mice deficient in both IL-1 ligands (IL-1α/β-/-), and in wild mice. The accumulation of neutrophils was used as a measure of the intensity of inflammation. It was demonstrated that LPS stimulated a significant increase in the accumulation of neutrophils in fetal fluid from the peritoneum, lungs and subcutaneous emphysema. Similar responses were observed in mice

with IL-1α/β-/-, which indicates that IL-1 actions are unnecessary to control cell recruitment in many extracerebral tissues. In contrast, the recruitment of neutrophils to the brain in response to an intrastriatic LPS injection was significantly weakened (by about 70%) in IL-1α/β-/- mice [53].

This data suggests fundamental differences in the requirement for IL-1 to regulate the key component of congenital brain immune response compared to extracerebral tissues. This is the 1st clear evidence that IL-1 controls neutrophilic encephalitis in a different way than in systemic tissues [53]. In addition, earlier studies showed that IL-1 deficiency [54] or the administration of IL-1RA [55] reduces the recruitment of neutrophils to the brain in response to brain ischemia. Therefore, these studies suggest that IL-1 is a key factor in the recruitment of innate immune cells to the brain in response to both microbiological ligands and sterile stimuli [53].

The fact that IL-1 is a key mediator of disorders in acute brain damage, especially in the case of a stroke, is also largely confirmed by endogenous IL-1 actions being blocked using IL-1RA and other IL-1 inhibitors, such as caspase-1 antibodies and inhibitors [1]. The first evidence of this, presented by Relton and Rothwell, shows that direct administration of IL-1RA to the rat brain can reduce injuries caused by experimental stroke or injection of excitotoxin [56]. A further study by Mulcahy et al. extended knowledge on the subject, showing that delayed administration of IL-1RA, up to 3 h from the onset of experimental stroke in rats, still had a protective function [57].

Moreover, a study conducted by Greenhalgh et al. showed that systemic administration of IL-1RA is as effective as central administration [58]. Importantly, treatment with IL-1RA also leads to a lasting improvement in post-stroke functional outcomes in rats [59]. Pharmacokinetic studies in animals and humans show that IL-1RA achieves a sustained therapeutic level in cerebrospinal fluid 8 h after systemic administration [58, 60]. Mice with IL-1α and IL-1β deficiency showed a marked decrease (80%) in brain damage after central cerebral artery occlusion [52], similar to animals with caspase-1 deficiency [61]. In a study conducted by Ross et al., a caspase-1 inhibitor (VRT-018858) also proved to be neuroprotective after experimental stroke in rats [62].

Several years ago, clinical trials showed that IL-1RA administered intravenously significantly weakened the systemic response of the acute phase of stroke without adverse effects [63]. So far, it is not clear how local and systemic inflammation contributes to brain damage, although a recent experiment in rodents suggests that both central and peripheral IL-1 derivatives are involved in ischemic brain damage [64]. This may be important because the available IL-1 inhibitors (IL-1RA and antibodies) are limited in their penetration into the brain and may act mainly by inhibiting peripheral IL-1 activities [1].

Interleukin-6

Interleukin-6 (IL-6) takes part in the pathogenesis of various pathological states, such as infection, autoimmunity, neurodegeneration and trauma [65].

In cellular communication, IL-6 is mediated by 2 distinct modes described as classical and trans-signal [66]. Classical signalling consists of the binding of IL-6 with a specific transonemic receptor called IL-6Rα [67]. Trans-signalisation, on the other hand, is based on the fact that the naturally occurring soluble form of IL-6Rα – sIL-6R (produced by the removal of IL-6Rα transmembrane-ectodomain or by alternative splicing of IL-6Rα mRNA) [68, 69, 70, 71] is associated with IL-6, and this complex triggers the response to IL-6 in cells that do not have IL-6Rα [72, 73].

Interleukin-6 / soluble IL-6 receptor activity counteracts the naturally occurring soluble form of glycoprotein 130 (gp130) [74]. This antagonist prevents trans-signalisation by binding the IL-6/sIL-6R complex, and this property is used to distinguish trans-signalisation from classical signalling [67]. In transgenic mice that overproduce gp130, trans-signalisation – but not classic IL-6 signalling – is significantly inhibited [75]. Although gp130 is expressed by almost all cells, including CNS cells, the surface expression of IL-6R is more limited [76].

Neurons and astrocytes have been shown to react well to IL-6/sIL-6R, but not to IL-6 itself, suggesting that trans-signalisation is essential to mediate the interaction of IL-6 with these cells [77, 78, 79]. Microglia, on the other hand, react well to IL-6 itself through classic signalling [80]. Recently, it was demonstrated that an injection of gp130 into mouse brain cells accelerated regeneration from endotoxemia induced by LPS and reduced microglia hyperexcitability to LPS in older mice, emphasizing the role of IL-6 trans-signalisation in these processes [81, 82]. However, much remains unknown about the role of trans-signalisation in mediating the pathogenic activities of IL-6 in CNS [67].

Nuclear transcription factor NF-κB

The nuclear factor κB (NF-κB) signal pathway, mediated by TLR4, plays an important role in the initiation of encephalitis in CNS diseases [83]. In addition, NF-κB activation leads to the transcription of many pro-inflammatory genes that encode cytokines, chemokines and enzymes, such as IL-1β, tumor necrosis factor α (TNF-α) and inducible nitric oxide synthase (iNOS), mediators involved in the development of secondary brain injury in hypoxia-ischaemia and inflammation [84].

Cyclooxygenase

Cyclooxygenase (COX), also known as prostaglandin H synthase, catalyzes the 1st stage of the synthesis of prostanoids containing prostaglandin (PG), prostacyclin and thromboxanes [85].

Cyclooxygenase shows 2 catalytic activities: COX activity which catalyzes the formation of prostaglandin G_2 (PGG_2) from arachidonic acid, and peroxidase activity which reduces PGG_2 to prostaglandin H_2 (PGH_2) [85]. External factors may influence each of them independently, as these 2 enzymatic activities occur in separate sites on the COX molecule [86]. In addition to the constitutive isoform (cyclooxygenase-1 – COX-1) present in practically all cell types, a 2nd inducible isoform called COX-2 was also identified [87]. Cyclooxygenase-2 is expressed

in several cell types in response to growth factors, cytokines and proinflammatory molecules. It is an isoform responsible mainly for the production of prostanoids in acute and chronic inflammatory states [85].

Cyclooxygenase-1 and COX-2 are coded by 2 different genes (located on human chromosome 9 and 1 respectively). The *COX-2* gene is characterized by the presence of the TATA box, and many binding sites for transcription factors in the promoter region, which are responsible for the regulation of COX-2 expression. Moreover, the long non-translatable region 3′ acts as a determinant of mRNA instability or as an inhibitor of translation, suggesting posttranscriptional control of COX-2 expression. The *COX-1* gene is a classic housekeeping gene, without the TATA box in its promoter. At the protein level, both isoforms show >60% homology in humans and rodents [85]. While functional sites are conservative, several important substitutions cause important conformational changes in the active site of both isoenzymes, which may explain the different sensitivities of COX-1 and COX-2 to specific inhibitors [88]. A very important difference between the isoforms is the 18-amino acid insert near the C-end of COX-2, which is not present in COX-1 and enables the production of specific antibodies [85].

The distribution of COX isoforms has been widely studied in rat and human tissues. In most tissues, COX-1 seems to be the only constitutive isoform [85]. However, in the cells of the brain, nuclei, kidneys and the macula lutea, both COX-1 and COX-2 are expressed under physiological conditions [86]. In the rat brain, COX-1 and COX-2 immunoreactivity are present in separate neural populations distributed in different areas of the cerebral cortex and hippocampus [85]. In other regions, such as the midbrain, pons and spinal cord, COX-1 immunoreactivity is predominant. In contrast, COX-2 mRNA in the rat brain was detected in granulosa cells of the dentate gyrus, pyramidal neurons in the hippocampus, piriform cortex, layers of surface cells of the neocortex, amygdala, and at low levels in the striatum, thalamus and hypothalamus [89, 90]. Similarly, mRNAs for COX-1 and COX-2 are present in several regions of the human brain, although COX-2 is the predominant isoform, particularly in the hippocampus [91, 92].

In the brain of mammals, COX-2 undergoes constitutive expression in specific neuronal populations under normal physiological conditions [85]. This "constitutive" neuronal expression of COX-2 is "dynamically" regulated because it is dependent on normal synaptic activity; it rapidly increases during convulsions or ischaemia and is reduced by glucocorticoids [89]. The dependence of COX-2 expression on natural excitatory synaptic activity, is supported by the presence of COX-2 immunoreactivity in distal dendrites and dendritic spines (which participate in synaptic signalling) and its exclusive location in excitatory glutamatergic neurons [85].

Indirect evidence for the contribution of COX-2 to synaptic plasticity has been obtained in recent years using COX inhibitors in synaptic plasticity models *in vivo* and *in vitro* [85]. It was demonstrated that COX-2 inhibitors, but not selective COX-1 inhibitors administered on a systemic basis, disturbed spatial memory in rats [93].

Despite evidence of the physiological role of COX-2 in brain development and functioning, COX-2 knockout mice do not show any serious anatomical abnormalities of the brain – the significant compensatory effects of COX-1 and, perhaps, of COX-2, cannot be excluded here. When considering the importance of COX-2 activity in brain diseases, 2 main aspects should be considered. First, COX-2 is expressed under normal conditions and contributes to basic brain functions such as synaptic activity, memory consolidation and functional hyperemia. Second, "neurological inflammation" is a much more regulated response than peripheral tissue inflammation [85]. In a study by Shiow et al., an immunohistochemical analysis (IHC) was performed using a collection of samples from a foetal human brain (27, 30 and 31 weeks of pregnancy) to determine whether COX-2 is normally expressed in the developing human brain [94].

In the 3 cases, IHC staining revealed COX-2 expression: in Iba1-positive microglia; in Nestin-positive radial glia; and CD34-positive endothelial cells in the cellular zone. Then, in order to further investigate the expression of COX-2 in the pathology of white matter in newborns, immunohistochemical studies were performed on subcortical samples of white matter from post-mortem samples of the cingulate cortex from newborns suffering from hypoxic-ischaemic encephalopathy. It was found that COX-2 expression was significantly elevated in reactive GFAP+ astrocytes of white matter. The number of GFAP1+ astrocytes and CD45+ immunological cells, which cause COX-2 expression in control cases and hypoxic-ischaemic encephalopathy, was calculated, and it was found that GFAP+ astrocytes showed a significantly higher total number and expression of COX-2. For comparison, CD45+ cells were unchanged in total number or COX-2 expression [94].

Despite intense research over the last decade, evidence of the direct role of COX-2 in neurodegenerative events is still controversial, and further experimental and clinical studies are needed to improve our knowledge of how and when COX-2 inhibition can be beneficial to patients suffering from inflammatory and degenerative neuropathologies [85].

Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is a proinflammatory mediator from arachidonic acid due to the activity of COX enzymes, and sends signals to the EP family of surface cell receptors [95]. Prostaglandin E_2 can be released by activated microglia and reactive astrocytes in the immature brain [96, 97, 98].

A study conducted by Shiow et al. showed that PGE_2 has a direct effect on the cultures of oligodendrocyte precursor cells (OPC) obtained from mice and young rats [94]. After treatment with T3 hormone, OPCs differentiate and express bone morphogenetic protein (MBP) marker, and the expression of immature OPC Nkx2.2 (homeobox protein Nkx2.2) markers decreases [99]. Treatment with PGE_2 also resulted in a strong and dose-dependent decrease in the expression of MBP induced by T3 in that experiment. It was confirmed that PGE_2 blocks OPC maturation by monitoring the permanent expression of the immature OPC marker, i.e. Nkx2.2. Thus, PGE_2 is a strong inhibitor of mouse and rat OPC maturation *in vitro*, and it was

also demonstrated that it does not affect the proliferation or survival of OPC [94].

Prostaglandin E_2 works through 4 receptors coupled with protein G: EP1-EP4. Previous studies have shown that EP1 is the dominant receptor in the oligodendrocyte line [100, 101]. To determine whether PGE_2 acts through EP1 to interfere with OPC maturation, both pharmacological and genetic approaches were used. It has been shown that ONO-8711 is an EP1 specific inhibitor [102]. Joint treatment with ONO-8711 reverses the effects of PGE_2 on the expression of MBP and maintenance of Nkx2.2. Simultaneously, similar results were observed for rat OPC culture in the presence of SC-51089 [103], another specific EP1 inhibitor. These results show that PGE_2 directly inhibits *in vitro* maturation of OPC by activating the EP1 receptor [94].

Prostaglandin E_2, which develops preferentially during the enzymatic activity of COX-2, but not during that of COX-1, may participate in synaptic plasticity through several mechanisms, including the modulation of adrenergic, noradrenergic and glutamatergic neurotransmission, and actin remodeling in the cytoskeleton, thus influencing the shape of spines and dendrites, and affecting the regulation of membrane excitability [104]. Moreover, COX-2 PGs are involved in coupling synaptic plasticity with cerebral blood flow, which suggests a weakening of blood flow through the cortex in response to COX-2 NS398 inhibitor [85]. The hyperemic response was also weakened in mice with a mutated gene without COX-2 [105].

Transforming growth factor β

The transforming grow factor β (TGF-β) family consists of at least 30 structurally related polypeptide growth factors, including TGF-βs, MBPs, growth differentiation factors (GDFs) and others. Members of the TGF-β family are synthesized and secreted in an inactive form bound to a large latent polypeptide [106]. These factors are activated after proteolytic digestion by various proprotein convertases, e.g. Furin (SPC1) and Paired basic Amino acid Cleaving Enzyme (SPC4/PACE4); this process serves as a mechanism of local protein activity control [107, 108].

Transforming growth factor β1, one of the members of the TGF-β family, is a multifunctional and versatile cytokine that occurs in virtually all body tissues [106]. Transforming growth factor β1 plays an important role in key biological reactions, both physiological and pathological, as well as in embryogenesis and adult life, such as extracellular matrix deposition, cell cycle control and immune response [109, 110, 111].

Transforming growth factor β1 is a homodimeric protein secreted in an inactive form, associated with latency associated peptide (LAP) [106]. After secretion, TGF-β1-LAP is proteolytically cut in an extracellular matrix, which leads to the release of the active form of TGF-β1, which diffuses and acts autocrinally or paracrinally as a soluble factor [112, 113]. Transforming growth factor β1 exerts its action by activating transmembrane protein serine/threonine kinase type II, and receptors: transforming growth factor-β receptor II (TβRII) and transforming growth factor-β receptor I (TβRI). Type III receptor (TβRIII), also known as beta-glycan, consists of a large extracellular domain with a short cytoplasmic tail that binds different members of the TGF-β family [106].

The receptor TβRIII plays an important role in the recruitment and presentation of ligands for type I and II receptors [106]. After binding a ligand to TβRII, this receptor promotes TβRI phosphorylation, which in turn phosphorylates the protein mediators of canonical cytoplasmic transcription factors, Smad2 and Smad3 (receptor-regulated Smads), which in turn bind to Smad4 (common mediator – Co-Smad), forming a protein complex that moves to the nucleus and controls the expression of the target *TGF-β1* genes [107, 114]. Smad6 and Smad7 (Smads inhibitor) mediate negative modulation of the TGF pathway through at least 4 mechanisms: (1) competition with R-Smads for receptor interactions or Co-Smads (2) targeting receptors for degradation (3) by triggering receptor dephosphorylation, and (4) facilitating endosomal localization of receptors, which may lead to their lysosomal degradation [106].

Transforming growth factor β1 also activates noncanonical signalling cascades. These pathways include several intracellular protein mediators such as RasGTPase, mitogen-activated protein kinase (MAPK/Erk), p38, phosphatidylinositol-3 kinase (PI3K/Akt), and c-jun-N-end kinase (JNK) [110, 115, 116, 117]. Although these pathways can be independently activated, both canonical and non-canonical pathways can be transactivated and participate synergistically in various events [114].

Transforming growth factor β1 and its receptors have been identified in the progenitor zones of the developing cerebral cortex, spinal cord and mid-brain [118, 119, 120, 121] and are widely distributed in various regions of the adult brain [122, 123, 124, 125]. The expression of TβRI and TβRII *in vitro* and *in vivo* has been demonstrated in neurons, astrocytes, oligodendrocytes, microglia and brain endothelial cells [122, 126, 127, 128, 129]. It has been demonstrated that TGF-β1 plays an essential role in neurogenesis [118, 130, 131, 132, 133], neuronal migration [118, 131], synapse formation [126, 134, 135], growth regulation [136], gliogenesis [115, 116, 119, 127, 137] and angiogenesis [128, 138, 139].

Pre- and neonatal exposure to Pb and inflammatory reaction in the brain

In a study conducted by Li et al., pregnant female mice were treated with lead acetate (PbAc) in drinking water at 3 different concentrations, 0.1%, 0.5% and 1%, resulting in low, moderate and high whole blood concentrations. Exposure began from the beginning of the pregnancy until weaning (i.e. 21 days after birth – PND_{21}) by [3]. A significant increase in Pb levels in the blood and hippocampus were observed in the groups exposed to Pb in comparison with the control group, with blood Pb levels dose-dependent. The study showed increased expression of IL-1β in the hippocampus in young rats.

The expression of IL-6 in the rat hippocampus was also significantly elevated in the groups compared to the control group.

This study shows that the immunoactivity of IL-1 and IL-6 was significantly increased in the hippocampus in the groups exposed to Pb [3]. Increased concentrations of these cytokines

observed after Pb exposure, signal the induction of mechanisms leading to an inflammatory cascade and indicate potential proinflammatory effects. Increased immunoactivity for IL-1 and IL-6 may contribute to the progression of brain damage in the young mice where the mothers were exposed to Pb, and also suggest the potential toxic mechanism of Pb action at an early stage.

Also, Kasten-Jolly et al. studied the effect of 0.1 mM PbAc on gene expression of some cytokines in the CNS. Young mice were exposed to Pb from the 8th day of pregnancy to 21 days after birth, i.e. during the period when the mothers received drinking water with 0.1 mM PbAc. Such exposure caused the level of Pb in the baby mice blood to increase to 15–20 μg/dL. A significant change induced by PbAc in the expression of *IL-6* and *TGF-β1* genes was observed among the studied cytokines. The expression of the *IL-6* gene was higher in each studied brain area compared to control, i.e. in the frontal cortex, cerebellum, hypothalamus, striatum, hippocampus and black matter [140].

Importantly, increased expression of IL-6 during brain development may adversely affect neuronal growth and differentiation through reactive gliosis (increased size and number of astrocytes and branched microglia), and may have the effect of activating NMDA receptors in neurons, causing over-activation and subsequent necrosis of nerve cells [140, 141, 142]. The study also showed that exposure to 0.1 mM PbAc did not affect the expression of IL-6 protein in the frontal cortex, cerebellum or hypothalamus, while a decreased expression of IL-6 was observed in the striatum, hippocampus and black matter. However, as indicated by the authors, the discrepancy between the levels of mRNA and IL-6 protein in different areas of the brain was probably caused by the protein detection method, which measured only the free form of IL-6 and not IL-6 bound to its receptor [140].

In the same study, the expression of *TGF-β1* genes in the group exposed to 0.1 mM PbAc was highest in the cortex. The levels of TGF-β1 protein were elevated in the frontal cortex and cerebellum and reduced in the black matter. Slightly decreased levels of TGF-β1 were also observed in the striatum, hippocampus and hypothalamus, but those changes did not differ significantly from the control group [140]. In the study by Wyss-Coray et al., the overproduction of TGF-β1 by astroglial cells resulted in the stimulation of inflammatory processes in the CNS of transgenic mice [143], which in combination with the results of studies conducted by Kasten-Jolly et al., confirm the role of Pb in inflammatory processes in the CNS on the expression of the *TGF-β1* gene.

Wei et al. studied the effect of different doses of Pb (25–100 μM) on COX-2 induction in glioma C6 cells in rat and mouse microglia BV2 cells, primary cultures of cerebral cortex neurons, neuronal stem cells (NSC) and RBE4 cells (brain endothelium). The results of these studies showed that Pb caused COX-2 induction in C6, BV2, primary cortical neuron culture and NSC. In RBE4 Pb cells (at doses greater than 50 μM), it caused only a slight increase in *COX-2* gene expression [144]. Previous studies of that team showed that when exposed to heavy metals such as mercury and arsenic, the *COX-2* gene is regulated by transcription factors such as NF-κB, activator protein 1 (AP-1) and NFAT [145, 146, 147, 148, 149].

Wei et al. studied the influence of Pb on the induction of transcription of *COX-2* gene in a mechanism mediated by transcription factors NF-κB, AP-1 and NFAT. In that study, only transcription factor NFAT was upregulated by exposure to Pb. In order to confirm the key role of NFAT transcription factor in the induction of *COX-2* gene transcription by Pb, the authors mutated NFAT binding sites within the *COX-2* gene promoter, which resulted in the abolition of *COX-2* gene transcription, thus confirming that NFAT plays a key role in Pb-induced *COX-2* gene transcription in glial cells. That observation may potentially lead to the use of molecular COX-2 inhibitors in attempts to eliminate the neurotoxic effects of Pb [144].

Strużyńska et al. showed increased production of proinflammatory cytokines and axon damage, with simultaneous activation of astrocytes after exposure of the immature rat brain to PbAc. Rats of both sexes aged 15 days and over were intraperitoneally injected with PbAc at a daily dose of 15 mg/kg (group Pb) or saline (control group) for 2 weeks. The administration regimen used in that experiment resulted in an increase in Pb blood levels similar to those typical for long-term exposure to Pb (3.3 μg/dL in control group and 30.8 μg/dL in Pb group). Strużyńska et al. also examined rats exposed to Pb in terms of their proinflammatory cytokine expression profile in the brain. In the forebrain and hippocampus, but not in the cerebellum, they detected increased expression of proinflammatory cytokines, but the profiles of cytokine secretion were different. The hippocampus showed a growth in IL-1β and TNF-α, while the forebrain cortex showed a growth in IL-6. An increase in IL-1β after the administration of Pb signals the induction of mechanisms leading to an inflammatory cascade and indicates the potential proinflammatory effects of Pb [150].

PROSPECTS FOR FURTHER RESEARCH

Future studies can place particular emphasis on conditions conducive to neuronal damage through cytokines [3]. Determination of specific changes in the CNS environment responsible for increased harmful effects of proinflammatory cytokines may also reveal new goals in the treatment of Pb neurotoxicity [3].

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