Original Article

The Novel Neurotoxic and Neuroimmunotoxic Capabilities of Aflatoxin B1 on the Nervous System: A Review

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ABSTRACT

Aflatoxins B1(AFB1) is an ancillary lethal metabolite archetypally spawned by Aspergillus flavus as well as A. parasiticus mostly found all over the world but more in tropic and humid regions. AFB1 has been isolated is almost all food products. The isolation of AFB1 in humans was demonstrated using human fluid like urine, serum as well as breast milk. Also, AFB1 was isolated in human placenta in pregnant women. ELISA is extremely sensitive in detecting AFB1. AFB1 was capable of compromising the activities of the blood-brain barrier (BBB). AFB1 was capable of triggering peripheral and central nervous degeneration. Acute central nervous system (CNS) symptoms like coma, cerebral edema as well as death has been observed in AFB1 exposure to the brain. Also, symptoms of brain destruction such as dullness, restlessness, muscle tremor, convulsions, loss of memory, epilepsy, idiocy, loss of muscle coordination, and abnormal sensations have been associated with deficiencies of these neurotransmitter during AFB1exposure. AFB1 was capable of influencing the end products of proteins as well as amino acid metabolism leading to hyper-ammonemia which easily cross the BBB to trigger the synthesis of glutamate neurotransmitters which are cytotoxic to the brain cells and causes encephalopathy. Glutathione (GHS) depletion resulted in destruction to critical cellular components such as DNA, lipids and proteins via the 8,9 epoxides of AFB1.This review therefore elucidates the novel neurotoxic and neuroimmunotoxic roles of AFB1 on the CNS.

INTRODUCTION

Aflatoxins B1(AFB1) is a subsidiary poisonous metabolite archetypally spawned by Aspergillus flavus as well as A. parasiticus [1-3]. These species of molds are routinely isolated in warm or tropical counties across the world [1,4]. AFB1 are currently the most ruinous carcinogens amongst all the various kinds of aflatoxins isolated [1,2]. Studies have shown that, ingestion of food contaminated with AFB1 often linked with central nervous system (CNS) disease like neuropathies, demyelinating disease as well as neurocognitive deficits [3,5,6]. Furthermore, AFB1 was capable of causing direct damage to the CNS [7]. One of the mechanisms via AFB1 caused damage to the brain was downregulation of Na^+, K^+-ATPase in the cerebrum which was established in rats [3,7]. Also, AFBI was able to modulate the concentrations of several biogenic amines in the brain of mice [3,8]. More so, the toxin was able to modify entire brain serotonin (5-HT) concentrations in chickens [3,9].

Additionally, introduction of AFB1 into the rat's brains for three weeks augmented brain levels of the lipoperoxidation indicators such as malondialdehyde as well as 4-hydroxyalkenals [3,10]. Nevertheless, continuing incorporation of aflatoxins into feeds for turkeys, cattle as well as chickens resulted in neurological signs and symptoms like anorexia, staggering gait, recurrent falls as well as adrift movements in circles [7]. AFB1 has also been implicated in hypothetical oxidative stress-associated mechanisms [11]. The toxin was able to modify protein kinase C (PKC) resulting in cancer because this kinase acts as a cancer faciliatory receptor [3,12]. This review therefore elucidates the novel neurotoxic as well as neuroimmunotoxic roles of AFB1 on the CNS.

DETECTION OF AFLATOXIN B1 IN HUMANS

The isolation of AFB1 in humans was demonstrated using human fluid like urine, serum as well as breast milk [13,14]. Nevertheless, no study has demonstrated the isolation of AFB1 in the cerebrospinal fluid (CSF) of humans. Several studies have investigated urine samples and confirmed a relationship between overall daily ingestion of AFB1

and excretion of AFM1[13, 15]. Also, urine samples were examined and the concentrations of AFB1-N7-guanine adduct was concurrent well with AFB1 ingestion [16]. Wild et al also indicated that, the levels of AFB1-N7-guanine in urine was maximum on the first two days after AFB1 ingestion [17].

Studies have demonstrated a correlation between AFB1-lysine and daily ingested AFB1 contamination and established that AFB1-lysine could be a biomarker [17,18]. Wild et al also indicated that, the levels of AFB1-lysine in serum was maximum from 2-3 month after exposure to AFB1[17]. AFM1, a key metabolite of AFB1 was also detected in milk during lactation, and numerous studies confirmed incidence of this metabolite in human milk [18,19]. Furthermore, AFB1 was also isolated in samples of umbilical cord blood, signifying they can cross the placenta [17,20].

The laboratory investigative modalities used in detecting AFB1-lysine adduct in serum includes radioimmunoassay, enzyme linked immunosorbent assay [17,19]. Also, purification with immunoaffinity columns followed by separation by high performance liquid chromatography (HPLC) as well as detection by fluorescence was utilized to detect AFB1[17,21]. ELISA is extremely sensitive in detecting AFB1, but it was less specific and displays higher levels of AFB1-lysine because of concurrent isolation of adducts from reactions with other amino acids as well as ingestion of aflatoxins of analogous structure, for example AFG1[17]. HPLC-fluorescence is specific for AFB1-lysine, but it is less sensitive for large scale detection of AFB1 during mass contaminations [21].

AFLATOXINS B1 AND NERVOUS SYSTEM

AFB1 usual gets into the gut via food contaminated with the toxin [22]. After ingestion of the toxin, it passes through the gut and finally absorbed into the blood stream via passive diffusion [22]. The most principal organ with high affinity for the toxin is the liver because it detoxifies most of toxins including AFB1[23]. When the toxin gets to the liver, it is transformed into its epoxide form by liver biotransformation enzymes [22,23]. The epoxide form exits the liver into the systemic circulation which then delivers it to almost all the body organs and cells most especially the brain, lymphocytes and the lungs [24]. The toxin is able to enter the systemic circulation because of its lipophilic nature [22,23]. AFB1 is capable of depleting myelin sheath of the nerves thereby exposing the nerves to injuries. Myelin sheath is a substance that shells the nerves [22].

Several studies have demonstrated that, AFB1 was capable of triggering aberrations in mitochondrial DNA of brain cells resulting in malfunctioning oxidative phosphorylation [25,26]. The defective oxidative injury often leads to destruction in key cellular macromolecules like DNA, lipids as well as proteins [25,26]. Studies have further proven that; cellular fatty acids are freely oxidized by reactive oxygen species (ROS) triggered by AFB1 to generate lipid peroxyl radicals which in turn proliferate into MDA. The resultant MDA interrelates with cellular DNA to form DNA-MDA which influences the generation of energy in the brain [25,26]. The ROS often triggers aging, chronic degenerative disorders, neuroinflammatory disorders as well as glioma [27].

AFB1 was detected in the brains of about 81% of children living in regions with high exposures during autopsies [22,24]. In neuroblastoma cells, it was established that AFB1 deregulate signal transduction actions [28]. Furthermore, AFB1 was capable of compromising the activities of the blood-brain barrier (BBB) [22]. It is proven that; the BBB is constituted by human brain microvascular endothelial cells (HBMEC) which are derivatives of the brain cells [22]. The cytotoxicity effect of AFB1 was capable of disrupting HBMEC which are key constituents of the BBB resulting in the destruction as well as a comprise of the BBB [22].

Furthermore, brain cells have high affinity for AFB1 because of its lipophilic nature tissue [7,24]. A body of evidence indicates that, AFB1 augmented β-glucuronidase and β-galactosidase during early stages of peripheral nervous system (PNS) as well as CNS degeneration in rats [7]. This means AFB1 is capable of triggering peripheral and central nervous degeneration. Furthermore, AFB1 was capable inducing neuropathy via these enzymes [7]. The levels of these enzymes in the cerebellum, cerebrum as well as medulla oblongata were elevated in an experiment involving rats with AFB1 toxins which subsequently developed neuropathies [22].

NERVOUS SYSTEM AFLATOXIN B1 SIGNALING PATHWAYS

Studies have shown that AFB1 directly binds to unknown receptors on neuronal cells leading to a dose as well as time-dependent reduction in neuronal cell numbers [1,7]. More studies are needed to identity these unknown receptors. Nevertheless, it has been established that, the generation of energy in the nervous systems was compromised by AFB1 via the inhibition of magnesium pump (Mg^{2+}-ATPase) (Figure 1) [7].

Souza et al demonstrated that, AFB1 was capable of modifying the energetic balance associated with ATP metabolism via the inhibition of a crucial enzyme of cellular homeostasis in cells with high as well as moderate energy needs like brain cells [1]. They indicated further that, the inhibition reduces ATP production as well as the interaction sites of ATP initiation and utilization with a resultant inhibition of cerebral sodium-potassium pump (Na^+, K^+-ATPase) activities [1]. Na^+, K^+-ATPase is a fundamental enzyme conscientious for the electrochemical gradient across cell membranes, preservation of membrane potential, cell capacity, cellular, as well as excitatory neurotransmitters [1,29]. The enzymes required for ATP generation are often perceived at the terminal phase of oxidative phosphorylation (Figure 1) [7].

Souza et al further demonstrated that, AFB1 was capable of blocking cerebral creatine kinase (CK) action [1]. Zala et al demonstrated that, ATP produced by CK is predominantly utilized by brain cells via the Na^+, K^+-ATPase (Figure 1) [30]. It was proven that, CK is extremely sensitive to free radicals such as hydrogen peroxide, peroxyl radical, superoxide anion as well as hydroxyl radicals. CK was also implicated in the oxidation of the thiol group of these free radicals [1, 30, 31]. CK is well-known as a fundamental regulator of cellular energy homeostasis in the brain which has enormous as well as altering energy needs [32]. CK was capable of generating
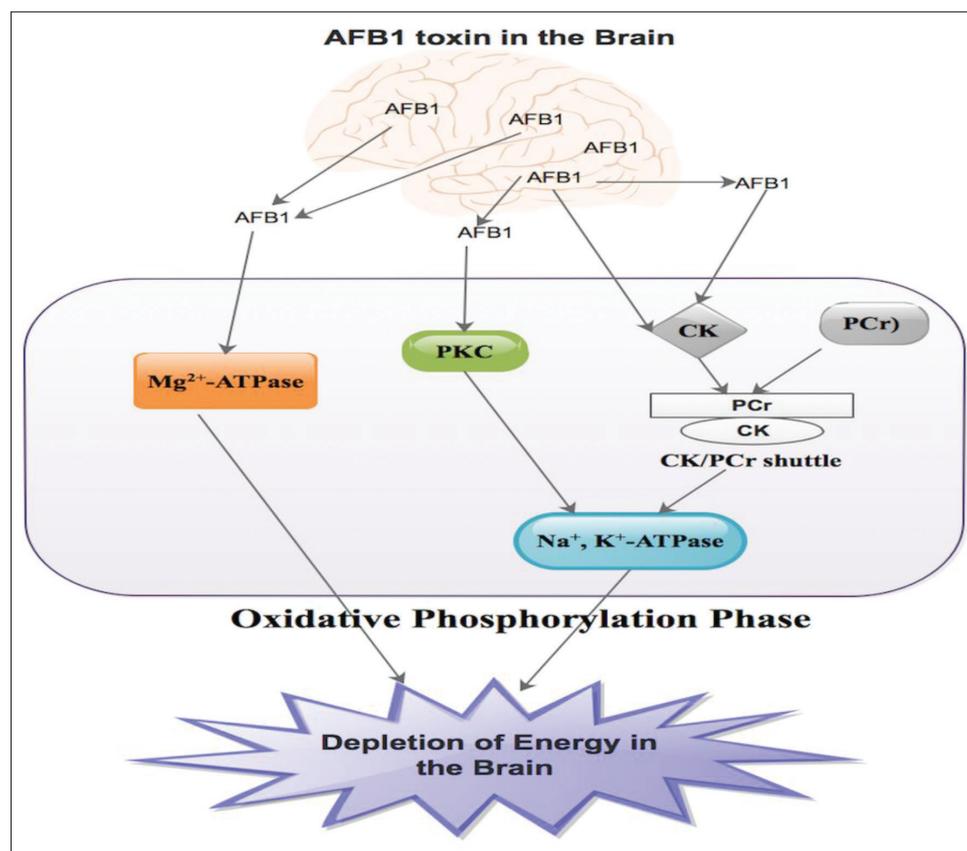
Figure 1. This an illustration showing the mechanisms via which AFB1 is able deplete energy in the brain. Abbreviations list: Aflatoxins B1 = AFB1, Creatine kinase = CK, Protein kinase C = PKC, Phosphocreatine = PCr

a huge pool of briskly circulating phosphocreatine (PCr) for temporal as well as spatial buffering of ATP concentrations via reversible transformation of creatine into PCr [32].

Schlattner et al indicated that, an energy transport system referred to as the CK/PCr shuttle or circuit is formed via the interconnections between CK and PCr (Figure 1) [33]. This energy transportation system shuttles energy from sub-cellular locations such as the mitochondria to locations of energy utilization, where the cellular ATPases as well as ATP-dependent ion pumps, like Na^+, K^+-ATPase are situated and wield a vital function in the appropriate role of this enzyme [33]. Venkataraman et al indicated that, CK activity was extremely susceptible to inactivation via oxidative reactions as well as oxidative damage, and thus one of the foci for ROS in cerebral diseases [34].

It has been proven that AFB1 contrarily modifies PKC activity in numerous nonneural cell types as well as neural cells [3,35]. Studies have also shown that, PKC signaling modulates Na^+, K^+-ATPase as well as oxidative stress in the brain (Figure 1) [36,37]. Activation of PKC was capable of influencing essential activities of neuronal physiology as well as regulatory processes in diverse subcellular compartments [3,38].

Studies have shown that, during the hepatic metabolism of AFB1, the detrimental consequences of AFB1 are intermediated by an augmented generation of reactive species like superoxide anion, hydroxyl radical, as well as hydrogen peroxide [3,39]. Studies have demonstrated that,

low dose of AFB1 was incapable of modifying the action of catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase as well as gluta-thione-S-transferase (GST) concentrations in brain [3,40]. Nevertheless, high dose of AFB1 had pro-oxidant influence in the brain [3]. The pro-oxidant effects lead to an augmented activity in SOD as well as a reduced GSH-Px activity in the brain, without contemporaneous modifications in MDA concentrations as well as CAT activity [3,41].

NEUROTRANSMITTERS AND AFLATOXIN B1

It has long been established that neurotransmitters as well as enzymes are the main substance that alters the behavior of all animals [3]. Studies have shown that food contaminated with AFB1 facilitates the pathological process of certain idiopathic as well as debilitating nervous diseases in humans [42,43]. AFB1 was capable of modifying the metabolism of tryptophan which in turn decrease the levels serotonin in the brain [44]. Also, AFB1 was able to lower the levels of striatal dopamine as well as serotonin which means that the toxin triggers dopaminergic as well as serotoninergic pathways via specific transformation of tyrosine to biogenic catecholamine neurotransmitters [45]. Furthermore, acute AFB1 contamination reduces brain acetylcholinesterase, while chronic exposure to the toxin up-regulates the levels of adenohypophyseal acetylcholinesterase [46].

Acute CNS symptoms like convulsions, coma, cerebral edema as well as death was observed during AFB1 exposure

to the brain [6]. Initial studies have demonstrated that, chronic exposure of AFB1 was capable of modulating cholinergic as well as dopaminergic transmission in the adult rat brain [46,47]. Furthermore, it was established that, AFB1 was able to modulate biogenic amine concentrations in some brain regions in a mouse exposed to AFB1-rich diet [6]. This modification was associated with transformations in the actions of some metabolic enzymes [8]. Also, AFB1 was implicated in the stimulation numerous histopathological modifications in the cerebral cortex as well as hippocampus in a rat model [48]. Moreover, maternal exposure to AFB1 as well as its metabolite AFM1 revealed crossing of these toxins from the bloodstream into milk and subsequently modifications in hippocampal neurogenesis with down-regulation of cholinergic signals in their progenies' [6,49].

It is well established that, AFB1 upregulates cholinesterases or acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), as well as adenosine deaminase (ADA) actions [50]. Up regulation of these enzymes during the AFB1 intoxication suggest the involvement of these enzymes in the pathological process [50]. Thus, ACh, BCh and ADA contribute to the physiological as well as pathological actions as inflammatory mediators and neurotransmission [50]. Furthermore, upregulations of AChE, BChE and ADA actions led to inflammation as well as tissue injury resulting in disease progression [50]. Also, modification in AChE and ADA actions may be associated with clinical signs of apathy, because these enzymes have significant contribution in neurotransmission as well as neuromodulation, correspondingly[50].

Acute exposure to AFB1 resulted in a reduction in regional brain AChE leading to alteration in cognitive functions, memory as well as impaired learning humans while chronic exposure resulted in an upsurge in adenohypophyseal AChE [23,51]. Studies have demonstrated that, absence of these neurotransmitter resulted in neurological symptoms like neurocognitive regression and modulation of sleep cycle [23,47]. Also, symptoms of brain destruction such as dullness, restlessness, muscle tremor, convulsions, loss of memory, epilepsy, idiocy, loss of muscle coordination, and abnormal sensations have been associated with deficiencies of these neurotransmitter during AFB1exposure [23]. AFB1 bind with guanine remains in DNA leading to AFB1-N7-guanine adducts and subsequently initiation of guanine to thiamine transversion mutations [23,52]. AFB1 also modifies the concentrations of numerous biogenic amines as well as their antecedents in rat and mouse brains [52].

ENCEPHALOPATHY AND DETOXIFICATION OF AFLATOXIN B1

It was well established that, the metabolism of AFB1 tonic effect starts from the live and often leads to liver failure as well as cancer of the liver [53]. Encephalopathy arises when the liver is incapable of detoxifying ammonia during failure or cancer as a result of AFB1 toxicity [54]. The end products of protein as well as amino acid metabolism often results in hyper-ammonemia which easily cross the BBB to trigger the synthesis of glutamate neurotransmitters which are cytotoxic to the brain cells leading to encephalopathy [53].

Cases of toxic encephalopathy in humans were first observed in Chinese children [55]. The involvement of AFB1 in toxic encephalopathy has also been observed in children with Reye's syndrome who were on salicylates [56]. AFB1 contamination resulting in Reye's syndrome with symptoms of encephalopathy as well as fatty degeneration of the viscera in humans who were not on salicylates have been reported [23,57]. Consequently, toxic encephalopathy associated with aflatoxicosis in dogs has also been reported [23]. Studies have shown that, AFB1 associated toxic encephalopathy often engrosses multiple symptoms such as recent memory decline, headaches, loss of balance, light headedness, insomnia, spaciness/disorientation, as well as loss of coordination [23,58].

Studies have shown that, AFB1 are metabolized via cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8,9-epoxide [59]. A sensitive type of the enzyme often adducts as well as damage DNA by binding to DNA as well as albumin in the blood serum [59, 60]. CYP3A4 as well as CYP1A2 are the principal human CYP450 isoforms implicated in the metabolism of AFB1 in humans [59]. However, CYP3A4 was the key CYP450 enzyme involved in the transformation of AFB1 into its epoxide type as well as AFQ1, a less toxic purification metabolite [59].

Glutathione (GSH) is an antioxidant that is capable of stabilizing as well as maintaining the cell membrane via decreasing oxidative stress and high ROS which stimulates lipid peroxidation [61]. Studies have shown that, AFB1 was capable of depleting GSH resulting in aberrantly high concentrations of ROS in cells [62,63]. The absence of GSH for GSH-peroxidase catalysis of O_2 to H_2O_2 often result in lipid peroxidation as well as cell membranes impairment [63]. GHS depletion often results in destruction to critical cellular components such as DNA, lipids and proteins via the 8,9 epoxides of AFB1[62].

Souto at al found that, AFB1 was capable of depleting key non-enzymatic soluble antioxidant defenses, ascorbic acid, as well as non- protein thiols in the cerebral cortex [3]. They explained that, AFB1 exposure modifies the brain redox state via non- protein thiols and ascorbic acid [3]. They specify that even a single exposure to AFB1 was capable of triggering acute imbalance in the brain non-enzymatic antioxidant defenses [3].

NEUROIMMUNOTOXIC EFFECTS AFB1

Studies have demonstrated that, murine microglial cells were able to trigger free radicals as well as inflammatory reactions during AFB1 contamination [64,65]. The cytotoxic consequence of AFB1 was perceived precisely at high levels on microglial cells in both time as well as dose-dependent manner [64]. Furthermore, ATP was significantly depleted in microglial cells contaminated with AFB1 using firefly luciferase assay [64]. Also, caspase-3/7 activities in AFB1-exposed microglia was significantly augments in bioluminescence assays which means that AFB1 is capable of influencing caspase-3/7 activity in microglia cells (Figure 2). Nevertheless, AFB1 was capable of triggering microglial apoptosis during microglial necrosis [64].

Gene expression analyses of human microglia demonstrated that, AFB1 has the ability initiate inflammatory response in human microglial cells at the mRNA level (Figure 2) [64]. Also, proinflammatory reactions associated with AFB1 toxin was implicated in the over-secretion of receptors such toll-like receptors (TLRs), myeloid differentiation primary response 88 (Myd88), nuclear factor kappa-light-chain-enhancer of activated B cells.

(NF-kB), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor.

(IKβ), C-X-C chemokine receptor type 4(CXCR4), C-C chemokine receptor type 4 (CCR4), and C-C chemokine receptor type 8 (CCR8) (Figure 2) [64].Nevertheless, AFB1 was unable to transform M_2 phenotype of microglial cells although the toxin is capable of triggering inflammatory response in the human microglial cells [64]. Therefore, microglial cells function as sensor of inflammation in the brain [66]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion in the AFB1-exposed microglia acts as danger signal for other resting microglial cells (Figure 2) [67]. This danger signaling modality occurs when GM-CSF binds to TLRs in microglia cells exposed to AFB1[67]. Also, augmentation of GM-CSF resulted in increased recruitment of inflammatory cells into the brain (Figure 2) [68].

A study demonstrated that, AFB1 was capable of modulating the expression of fundamental mRNAs as well as proteins associated with inflammation in essential immune-keeper cells of the microglial lineage in the brain [65]. These immune-keeper cells of microglial cell induce neurotoxic astrocytes [64,69]. The destructive consequences of AFB1 on human microglia was often due to ATP depletion as well as the induction of apoptosis [64]. These reactions automatically elucidate the hypothetical warning of neuro-immune dysregulation by AFB1 on the human brain [64,65].

Park et al in a recent study indicated that, AFB1 treatment triggered mitochondria-dependent apoptosis of the human astrocytes, NHA-SV40LT cells [70]. They stated that, AFB1 triggered cytosolic and mitochondrial calcium changes as well as ROS production in the cells above [70]. AFB1 was capable of modulating the proliferative abilities of the NHA-SV40LT cells via the AKT and ERK1/2 MAPK signaling [70]. Also, AFB1 blocked the sustainability as well as growth of zebrafish embryo via the triggering of casp3a, casp8, casp9, and tp53 [70]. AFB1 was capable of triggering apoptosis in astrocytes as well as oligodendrocytes in the brain and axon of the zebrafish embryo [70]. Thus, AFB1 stimulated cell death in astrocytes as well as blocked general brain growth [70].
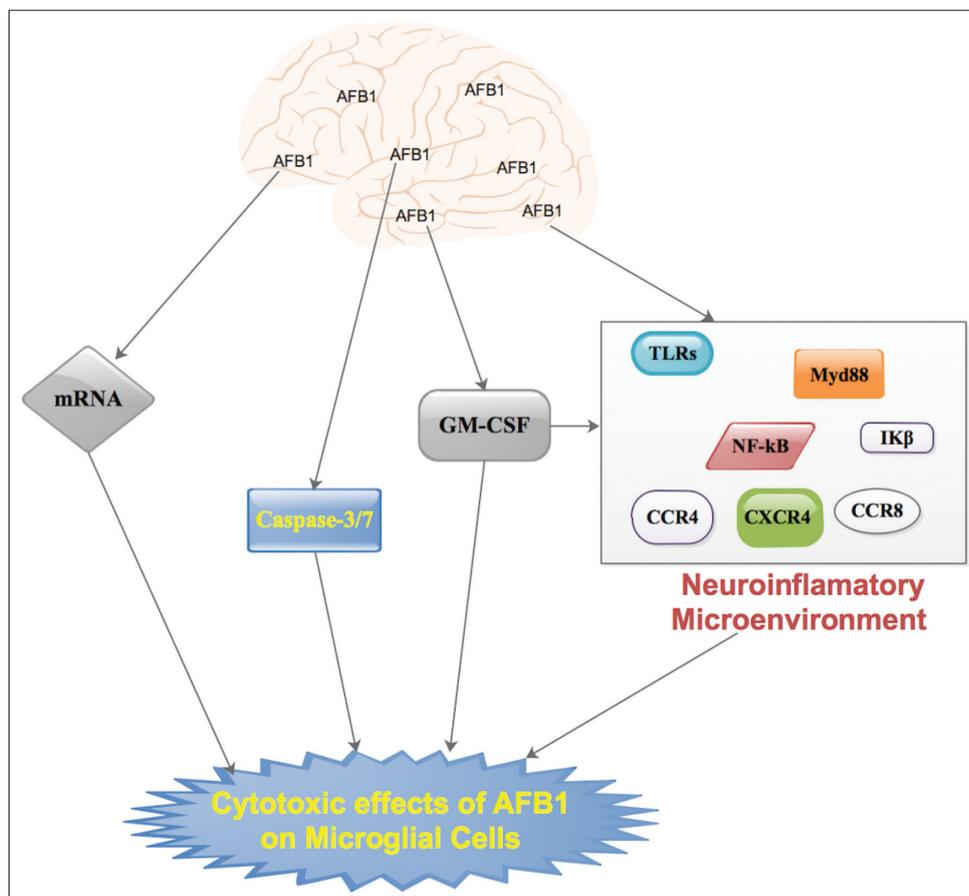

Figure 2. This an illustration showing the cytotoxic effect of AFB1 on microglial cells. Abbreviations list: Aflatoxins B1 = AFB1, C-X-C chemokine receptor type 4 = CXCR4, C-C chemokine receptor type 4 = CCR4, C-C chemokine receptor type 8 = CCR8, Granulocyte-macrophage colony-stimulating factor = GM-CSF, Myeloid differentiation primary response 88 = Myd88, Nuclear factor kappa-light-chain-enhancer of activated B cells = NF-kB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor = Ikβ, Toll-like receptors =TLRs

CONCLUSION

Ingestion of food contaminated with AFB1 is associated with CNS disease like neuropathies, demyelinating disease as well as neurocognitive deficits. Also, acute CNS symptoms like convulsions, coma, cerebral edema as well as death has been observed in AFB1 exposure to the brain. AFB1interacts with protein as well as amino acid metabolism resulting in hyper-ammonemia which easily cross the BBB to trigger the synthesis of glutamate neurotransmitters which are cytotoxic to the brain cells leading to encephalopathy. Furthermore, GHS depletion results in destruction to critical cellular components such as DNA, lipids and proteins via the 8,9 epoxides of AFB1. The destructive consequences of AFB1 on human microglia was often due to ATP depletion as well as the induction of apoptosis. These reactions automatically elucidate the hypothetical warning of neuroimmune dysregulation by AFB1 on the human brain. Further signaling (interleukins) studies on the effect of AFB1 on the nervous system is warranted.

ABBREVIATIONS LIST

Acetylcholinesterase = AchE, Adenosine deaminase = ADA, Aflatoxins B1 = AFB1, Blood-brain barrier = BBB, Butyrylcholinesterase = BChE, Creatine kinase = CK, catalase = CAT, cytochrome P450 = CYP450, C-X-C chemokine receptor type 4 = CXCR4, C-C chemokine receptor type 4 = CCR4, C-C chemokine receptor type 8 = CCR8, Glutathione = GHS, glutathione-S-transferase =GST, Granulocyte-macrophage colony-stimulating factor = GM-CSF, High performance liquid chromatography = HPLC, Human brain microvascular endothelial cells = HBMEC, Protein kinase C = PKC, Malondialdehyde = MDA, Myeloid differentiation primary response 88 = Myd88, Nuclear factor kappa-light-chain-enhancer of activated B cells = NF-kB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor = Ikβ, Peripheral nervous system = PNS, Phosphocreatine = PCr, Reactive oxygen species = ROS, Superoxide dismutase = SOD, Toll-like receptors =TLRs.

AUTHOR CONTRIBUTIONS

All authors contributed toward literature search, drafting and critically revision of the paper and agree to be accountable for all aspects of the work.

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REFERENCE

1. Souza CF, Baldissera MD, Zeppenfeld CC, Descovi S, Stefani LM, Baldisserotto B, et al. Oxidative stress mediated the inhibition of cerebral creatine kinase activity in silver catfish fed with aflatoxin B 1-contaminated diet. Fish physiology and biochemistry. 2019;45:63-70.

2. Hussain D, Mateen A, Gatlin III DM. Alleviation of aflatoxin B1 (AFB1) toxicity by calcium bentonite clay: Effects on growth performance, condition indices and bioaccumulation of AFB1 residues in Nile tilapia (Oreochromis niloticus). Aquaculture. 2017;475:8-15.

3. Souto NS, Claudia Monteiro Braga A, Lutchemeyer de Freitas M, Rechia Fighera M, Royes LFF, Schneider Oliveira M, et al. Aflatoxin B1 reduces non-enzymatic antioxidant defenses and increases protein kinase C activation in the cerebral cortex of young rats. Nutritional neuroscience. 2018;21:268-75.

4. Benkerroum N. Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. International journal of environmental research and public health. 2020;17:423.

5. Shyamal S, Latha P, Suja S, Shine V, Anuja G, Sini S, et al. Hepatoprotective effect of three herbal extracts on aflatoxin B1-intoxicated rat liver. Singapore medical journal. 2010;51:326.

6. Scafuri B, Varriale A, Facchiano A, D'Auria S, Raggi ME, Marabotti A. Binding of mycotoxins to proteins involved in neuronal plasticity: a combined in silico/wet investigation. Scientific reports. 2017;7:15156.

7. Ikegwuonu FI. The neurotoxicity of aflatoxin B1 in the rat. Toxicology. 1983;28:247-59.

8. Jayasekara S, Drown D, Coulombe R, Sharma R. Alteration of biogenic amines in mouse brain regions by alkylating agents. I. Effects of aflatoxin B 1 on brain monoamines concentrations and activities of metabolizing enzymes. Archives of environmental contamination and toxicology. 1989;18:396-403.

9. Ahmed K, Zieve L, Quarfoth G. Effects of methanethiol on erythrocyte membrane stabilization and on Na+, K+-adenosine triphosphatase: relevance to hepatic coma. Journal of Pharmacology and Experimental Therapeutics. 1984;228:103-8.

10. Gesing A, Karbownik-Lewinska M. Protective effects of melatonin and N-acetylserotonin on aflatoxin B1-induced lipid peroxidation in rats. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. 2008;26:314-9.

11. Mistry K, Krishna M, Bhattacharya R. Effect of aflatoxin B1 on phosphoinositide signal transduction pathway during regeneration of liver cells following partial hepatectomy. 2001.

12. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature. 1984;308:693.

13. Zhu J-q, Zhang L-s, Hu X, Xiao Y, Chen J-s, Xu Y-c, et al. Correlation of dietary aflatoxin B1 levels with excretion of aflatoxin M1 in human urine. Cancer research. 1987;47:1848-52.

14. Jager AV, Ramalho FS, Zambelli LN, Oliveira CAF. Biomarkers of aflatoxin exposure and its relationship with the hepatocellular carcinoma. Aflatoxins-Biochemistry and Molecular Biology: IntechOpen; 2011.

15. Groopman JD, Jiaqi Z, Donahue PR, Pikul A, Lisheng Z, Jun-shi C, et al. Molecular dosimetry of urinary aflatoxin-DNA adducts in people living in Guangxi Autonomous Region, People's Republic of China. Cancer research. 1992;52:45-52.

16. Groopman JD, Hall AJ, Whittle H, Hudson GJ, Wogan GN, Montesano R, et al. Molecular dosimetry of aflatoxin-N7-guanine in human urine obtained in The Gambia, West Africa. Cancer Epidemiology and Prevention Biomarkers. 1992;1:221-7.

17. Wild C, Rasheed F, Jawla M, Hall A, Jansen L, Montesano R. In-utero exposure to aflatoxin in West Africa. Lancet. 1991;337:1602.

18. Wild CP, Jiang Y-Z, Sabbioni G, Chapot B, Montesano R. Evaluation of methods for quantitation of aflatoxin-albumin adducts and their application to human exposure assessment. Cancer Research. 1990;50:245-51.

19. Gan L-S, Skipper PL, Peng X, Groopman JD, Chen J-s, Wogan GN, et al. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B 1 intake and urinary excretion of aflatoxin M 1. Carcinogenesis. 1988;9:1323-5.

20. Turner PC, Collinson AC, Cheung YB, Gong Y, Hall AJ, Prentice AM, et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. International journal of epidemiology. 2007;36:1119-25.

21. Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, et al. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. International journal of cancer. 1996;67:620-5.

22. Qureshi H, Hamid SS, Ali SS, Anwar J, Siddiqui AA, Khan NA. Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. Medical mycology. 2015;53:409-16.

23. Bbosa GS, Kitya D, Lubega A, Ogwal-Okeng J, Anokbonggo WW, Kyegombe DB. Review of the biological and health effects of aflatoxins on body organs and body systems. Aflatoxins-recent advances and future prospects. 2013;12:239-65.

24. Oyelami O, Maxwell S, Adelusola K, Aladekoma T, Oyelese A. Aflatoxins in the autopsy brain tissue of children in Nigeria. Mycopathologia. 1995;132:35-8.

25. Wang H, Dick R, Yin H, Licad-Coles E, Kroetz DL, Szklarz G, et al. Structure− function relationships of human liver cytochromes P450 3A: aflatoxin B1 metabolism as a probe. Biochemistry. 1998;37:12536-45.

26. Uetsuka K. Mechanisms of mycotoxin-induced neurotoxicity through oxidative stress-associated pathways. International journal of molecular sciences. 2011;12:5213-37.

27. Halliwell B. Oxidative stress and cancer: have we moved forward? Biochemical Journal. 2006;401:1-11.

28. Bonsi P, Augusti-Tocco G, Palmery M, Giorgi M. Aflatoxin B1 is an inhibitor of cyclic nucleotide. General Pharmacology: The Vascular System. 1999;32:615-9.

29. de Lores Arnaiz GR, Ordieres MGL. Brain Na+, K+-ATPase activity in aging and disease. International journal of biomedical science: IJBS. 2014;10:85.

30. Zala D, Hinckelmann M-V, Yu H, Da Cunha MML, Liot G, Cordelières FP, et al. Vesicular glycolysis provides on-board energy for fast axonal transport. Cell. 2013;152:479-91.

31. Glaser V, Leipnitz G, Straliotto MR, Oliveira J, dos Santos VV, Wannmacher CMD, et al. Oxidative stress-mediated inhibition of brain creatine kinase activity by methylmercury. Neurotoxicology. 2010;31:454-60.

32. Schlattner U, Tokarska-Schlattner M, Wallimann T. Mitochondrial creatine kinase in human health and disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2006;1762:164-80.

33. Schlattner U, Klaus A, Rios SR, Guzun R, Kay L, Tokarska-Schlattner M. Cellular compartmentation of energy metabolism: creatine kinase microcompartments and recruitment of B-type creatine kinase to specific subcellular sites. Amino Acids. 2016;48:1751-74.

34. Venkataraman P, Krishnamoorthy G, Selvakumar K, Arunakaran J. Oxidative stress alters creatine kinase system in serum and brain regions of polychlorinated biphenyl (Aroclor 1254)-exposed rats: protective role of melatonin. Basic & clinical pharmacology & toxicology. 2009;105:92-7.

35. den Heever Van L, Dirr H. Effect of aflatoxin B1 on human platelet protein kinase C. The International journal of biochemistry. 1991;23:839-43.

36. Pedemonte CH, Pressley TA, Lokhandwala MF, Cinelli AR. Regulation of Na, K-ATPase transport activity by protein kinase C. The Journal of membrane biology. 1997;155:219-27.

37. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress. Free Radical Biology and Medicine. 2000;28:1349-61.

38. Obis T, Hurtado E, Nadal L, Tomàs M, Priego M, Simon A, et al. The novel protein kinase C epsilon isoform modulates acetylcholine release in the rat neuromuscular junction. Molecular brain. 2015;8:80.

39. Towner RA, Qian SY, Kadiiska MB, Mason RP. In vivo identification of aflatoxin-induced free radicals in rat bile. Free Radical Biology and Medicine. 2003;35:1330-40.

40. Yener Z, Celik I, Ilhan F, Bal R. Effects of Urtica dioica L. seed on lipid peroxidation, antioxidants and liver pathology in aflatoxin-induced tissue injury in rats. Food and Chemical Toxicology. 2009;47:418-24.

41. Kanbur M, Eraslan G, Sarıca ZS, Aslan Ö. The effects of evening primrose oil on lipid peroxidation induced by subacute aflatoxin exposure in mice. Food and Chemical Toxicology. 2011;49:1960-4.

42. Olson LC, Bourgeois CH, Cotton RB, Harikul S, Grossman RA, Smith TJ. Encephalopathy and fatty degeneration of the viscera in northeastern Thailand. Clinical syndrome and epidemiology. Pediatrics. 1971;47:707-16.

43. Fung F, Clark RF. Health effects of mycotoxins: a toxicological overview. Journal of Toxicology: Clinical Toxicology. 2004;42:217-34.

44. Kimbrough T, Llewellyn G, Weekley L. The effect of Aflatoxin B 1 exposure on serotonin metabolism: Response to a tryptophan load. Metabolic brain disease. 1992;7:175-82.

45. Coulombe Jr RA, Sharma R. Clearance and excretion of intratracheally and orally administered aflatoxin B1 in the rat. Food and chemical toxicology. 1985;23:827-30.

46. Egbunike GN, Ikegwuonu FI. Effect of aflatoxicosis on acetylcholinesterase activity in the brain and adenohypophysis of the male rat. Neuroscience letters. 1984;52:171-4.

47. Coulombe Jr RA, Sharma RP. Effect of repeated dietary exposure of aflatoxin B1 on brain biogenic amines and metabolites in the rat. Toxicology and applied pharmacology. 1985;80:496-501.

48. Bahey NG, Elaziz HOA, Gadalla KKES. Toxic effect of aflatoxin B1 and the role of recovery on the rat cerebral cortex and hippocampus. Tissue and cell. 2015;47:559-66.

49. Tanaka T, Mizukami S, Hasegawa-Baba Y, Onda N, Sugita-Konishi Y, Yoshida T, et al. Developmental exposure of aflatoxin B1 reversibly affects hippocampal neurogenesis targeting late-stage neural progenitor cells through suppression of cholinergic signaling in rats. Toxicology. 2015;336:59-69.

50. da Silva AS, Santurio JM, Roza LF, Bottari NB, Galli GM, Morsch VM, et al. Aflatoxins produced by Aspergillus parasiticus present in the diet of quails increase the activities of cholinesterase and adenosine deaminase. Microbial pathogenesis. 2017;107:309-12.

51. Coulombe Jr RA. Nonhepatic disposition and effects of aflatoxin B1. The Toxicology of Aflatoxins: Elsevier; 1994. p. 89-101.

52. Lopez C, Ramos L, Bulacio L, Ramadan S, Rodriguez F. Aflatoxin B1 content in patients with hepatic diseases. MEDICINA-BUENOS AIRES-. 2002;62:313-6.

53. Bémeur C, Desjardins P, Butterworth RF. Role of nutrition in the management of hepatic encephalopathy in end-stage liver failure. Journal of nutrition and metabolism. 2010;2010.

54. Butterworth RF. Complications of cirrhosis III. Hepatic encephalopathy. Journal of hepatology. 2000;32:171-80.

55. Lye MS, Ghazali AA, Mohan J, Alwin N, Nair RC. An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia. The American journal of tropical medicine and hygiene. 1995;53:68-72.

56. Dvorackova I, Kusak V, Veselý D, Vesela J, Nesnidal P. Aflatoxin and encephalopathy with fatty degeneration of viscera (Reye). Annales de la nutrition et de l'alimentation1977. p. 977-89.

57. Ryan NJ, Hogan GR, Hayes AW, Unger PD, Siraj MV. Aflatoxin B1: its role in the etiology of Reye's syndrome. Pediatrics. 1979;64:71-5.

58. Cauli O. Brain Aquaporin 4 in Hyperammonemia. Medicina universitaria. 2010;12:47-53.

59. Wild CP, Montesano R. A model of interaction: aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. Cancer letters. 2009;286:22-8.

60. Wu F, Khlangwiset P. Health economic impacts and cost-effectiveness of aflatoxin-reduction strategies in Africa: case studies in biocontrol and post-harvest interventions. Food Additives and Contaminants. 2010;27:496-509.

61. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2001;482:21-6.

62. Johnson WW, Ueng Y-F, Widersten M, Mannervik B, Hayes JD, Sherratt PJ, et al. Conjugation of highly reactive aflatoxin B1 exo-8, 9-epoxide catalyzed by rat and human glutathione transferases: estimation of kinetic parameters. Biochemistry. 1997;36:3056-60.

63. Stewart RK, Serabjit-Singh CJ, Massey TE. GlutathioneS-Transferase-Catalyzed Conjugation of Bioactivated Aflatoxin B1in Rabbit Lung and Liver. Toxicology and applied pharmacology. 1996;140:499-507.

64. Mehrzad J, Hosseinkhani S, Malvandi AM. Human Microglial Cells Undergo Proapoptotic Induction and Inflammatory Activation upon in vitro Exposure to a Naturally Occurring Level of Aflatoxin B1. Neuroimmunomodulation. 2018;25:176-83.

65. Mehrzad J, Malvandi AM, Alipour M, Hosseinkhani S. Environmentally relevant level of aflatoxin B1 elicits toxic pro-inflammatory response in murine CNS-derived cells. Toxicology letters. 2017;279:96-106.

66. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends in neurosciences. 1996;19:312-8.

67. Parajuli B, Sonobe Y, Kawanokuchi J, Doi Y, Noda M, Takeuchi H, et al. GM-CSF increases LPS-induced production of proinflammatory mediators via upregulation of TLR4 and CD14 in murine microglia. Journal of neuroinflammation. 2012;9:268.

68. Vogel DY, Kooij G, Heijnen PD, Breur M, Peferoen LA, van der Valk P, et al. GM-CSF promotes migration of human monocytes across the blood brain barrier. European journal of immunology. 2015;45:1808-19.

69. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541:481.

70. Park S, Lee J-Y, You S, Song G, Lim W. Neurotoxic effects of aflatoxin B1 on human astrocytes in vitro and on glial cell development in zebrafish in vivo. Journal of hazardous materials. 2020;386:121639.