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論文内容の要旨

People are exposed to variety of environmental electrophiles such as acrylamide (ACR) in baked foods, methylmercury accumulated in fish, cadmium in rice, aromatic hydrocarbon quinones formed during gasoline combustion or crotonaldehyde in tobacco smoke. The mechanism how these electrophiles induce toxicity is thought to modify nucleophilic functions in proteins, such as cysteine residues, forming adducts and activating cellular redox signal transduction pathways such as kinases and transcription factors. Higher concentrations of electrophiles, on the other hand, disrupt such signaling by nonselective covalent modification of cellular proteins leading to eventually oxidative and inflammatory response which may be the cause of the neurotoxicity and or neurodegeneration. This work focus on the ACR and (B[a]P) neurotoxicity in mice.

Acrylamide (ACR) is, a white, odorless, and water-soluble substance, a soft electrophile which is known as a potent neurotoxicant in humans. It is generated during processing food at high temperatures through the Maillard reaction. There are different sources of ACR including fried, baked potatoes, coffee and bakery products contain substantial

amounts of acrylamide. It is also used in water sanitation and production flocculators, grouts, and press fabrics. Besides, chemical monomer acrylamide is used to produce polymer compounds that are utilized to make plastic, sheets, adhesive tapes, colors, and food packaging. Therefore, humans are exposed to ACR through different sources in addition to occupationally exposure. In humans and experimental animals, acrylamide is a known neurotoxicant that causes neuropathies and encephalopathies. Inflammatory cytokines play an important role in the inflammatory response of the body against stress, damage, or disease. One of the important proinflammatory cytokines is interleukin-1β (*IL-1β).* It is secreted from microglia and considered a critical neuroinflammatory component of brain reaction to any insults, but its role in electrophile-induced neurotoxicity is not well understood.

Benzo[a]pyrene (B[a]P) is the most common polycyclic aromatic hydrocarbon (PAH) found in the atmosphere, surface water, soil, and sediments. It is present in cigarette smoke as well as food products, particularly when smoked and grilled, so humans are exposed to B[a]P from multiple sources. B[*a*]P and other PAHs have been shown to be formed during the burning of fossil fuels, wood, and other organic materials. B[*a*]P has been detected at high levels in cigarette smoke, diesel exhaust, charcoal-based foods, as well as industrial wastes. The main sources of human B[*a*]P exposure are contaminated food and the air. Occupational exposure also to B[*a*]P, occurs mainly through inhalation or dermal absorption through combustion of PAHs mixture at workplaces. There are several studies demonstrating that these electrophiles act through increase in ROS formation and induced imbalance in the biological oxidant to antioxidant ratio leading to oxidative stress and alteration of antioxidant defense system which causes subsequently DNA damage or neurological impairments such as neuronal loss, glial activation or neuroinflammation.

This thesis aims to clarify the mechanisms which may be the cause of neurotoxicity or neurodegeneration caused by environmental electrophiles. The first study aims to investigate the role of *IL-1β* in ACR-induced neurotoxicity in mice as *IL-1β* one of cytokines that released in response to any harmful stimuli to relive inflammatory response. The second study aims to investigate the underlying mechanisms and histopathological changes in mouse brain in relation to B[*a*]P-induced neurotoxicity in mice.

In the first study, ten weeks old male wild type (C57BL/6JJmsSLC) and *IL-1β* knock-out mice were allocated into three groups of 10 each and exposed to ACR 0, 12.5, 25 mg/kg body weight by oral gavage for 7 days/week for 28 days. Compared to wild type mice, the result revealed, a significant increase in landing foot spread test in *IL-1β* KO mice exposed to ACR at high dose and decrease in density of noradrenergic axon in somatosensory area. In wild type mice, *IL-1β* induced a significant increase in expression of *Nrf2* gene and its downstream antioxidant (*HO-1*), (*NQO1*), (*GST-M*)) as well as *IL-6* and increased *NF-kB1* expression. The *IL-1β* protected the mice brain against ACR-induced neurotoxicity. The mechanism of protection might be through suppression of oxidative stress, upregulation of *NrF2/NF-kB1* pathway or *IL-6,* or *TNF-a* and *IL-1β* synergistic effect. This unexpected result gives new insight about the fundamental role of *IL-1β* in ACR-induced neurotoxicity in vivo.

We analyzed bulk RNA-seq data of murine cerebral cortex (18 mice, C57BL/6JJmsSLC) after exposure to ACR for 28 days. The identified expression profiles were shown to be correlated with pathways of multiple neurodegenerative diseases, as evidenced by enrichment results of Gene Ontology/KEGG Pathway items. Upregulation of various proteasome subunits, such as Psma3, Psma5, Psma7, Psmb1, Psmb2, Psmb3, Psmb6, Psmc2, Psmc4, and Psmd14, was found in ACR-exposed murine brain.

In the second study, we used forty, ten weeks old, male mice (C57BL/6JJcl), which were allocated into 4 groups (each contain 10 animal) and exposed to B[*a*]P at doses 0, 2.88, 8.67, 26.00 μg/mice respectively, by pharyngeal aspiration once/week for 4 weeks. The effects of B[*a*]P on motor function were assessed by landing foot spread test (LFS), short-term memory and anxiety like behavior were evaluated by using Y-maze and elevated plus maze test. Noradrenergic and serotonergic axon density was evaluated by IHC in hippocampal CA1 and CA3 area. The quantitative RT PCR was carried out for inflammatory cytokines, Nrf2 and downstream genes, B[*a*]P metabolism and DNA repair genes. The result showed no alteration on body weight gain throughout the treatment, while motor function (LFS) declined in all treated groups in third and fourth weeks. The density of noradrenergic axons significantly decreased in CA3 area of hippocampus (low and middle groups compared to control) and CA1 area for all treated groups compared to control. The density of serotonergic axons significantly decreased in CA1 and CA3 area of hippocampus at high dose compared to other groups. The expression analysis showed

no alteration in *Nrf2* and down genes *HO-1, SOD-1, GST5M, GSTM4*. However, B[*a*]P treatment significantly enhances the expression of inflammatory cytokines including *TNF-a*, *IL-1β*, *IL-6*, *IL-18* and *NLRP3,* and significantly increased expression of *CYP1A1* gene which was involved in B[*a*]P metabolism. These finding data suggest that exposure to BaP induces neurotoxicity in mice, which may be mediated by inflammatory pathway rather than oxidative stress pathway.

Key Words: Acrylamide, Benzo[a]pyrene, *IL-1β,* Neurotoxicity; Noradrenergic axons, Serotonergic axon, *NRf2*, Neuroinflammation, Transcriptome.

論文審査の結果の要旨

環境中には様々な親電子性物質あるいは代謝によって親電子性物質に変換される前親電子性物質が存在する。疫学研究は親電子性物質 1-ブロモプロパン、アクリルアミドへの曝露によるヒトの中枢神経障害、前親電子性物質ベンゾピレンへの曝露による労働者の行動変化を示している。本研究では、最初に前親電子性物質ベンゾピレンへの曝露がマウス脳におけるノルアドレナリンおよびセロトニン作動性神経に及ぼす影響を明らかにした。10 週齢雄マウスにベンゾピレンを経気道的に週 1 回、4 週曝露した。その結果、ベンゾピレンへの曝露はマウス脳海馬 CA1 および CA3 領域におけるノルアドレナリン作動性神経およびセロトニン作動性神経の密度を量依存的に減少させることが明らかとなった。さらに炎症促進サイトカイン遺伝子または炎症関連遺伝子 *Tnfα*、*Il-1β* 、*Il-18* 、*Nlrp3* の発現を上方制御した。この結果はベンゾピレンがノルアドレナリンおよびセロトニン作動性神経の変性を誘導することを示すとともに、神経変性に炎症関連遺伝子が関連していることを示唆した。次に炎症促進サイトカインである interleukin-1β (IL-1β) が親電子性物質による中枢神経障害に果たす役割を明らかにするために *IL-1β* ノックアウトマウスを用いて検討した。その結果、IL-1β の欠損は予想と反してアクリルアミドの中枢神経毒性を増強し、IL-1β が神経保護的に働くこととがわかった。野生型マウスで見られるアクリルアミド曝露によるグルタチオン産生酵素とグルタチオンペルオキシダーゼ遺伝子の上方制御が IL-1β の欠損によって消失することから、IL-1β の神経保護作用はグルタチオン代謝によるものであることが示唆された。

審査では１）ベンゾピレンの代謝経路、２）ベンゾピレンによる影響とアクリルアミドによる影響との違いと共通性、３）肺炎症と神経炎症との関係、４）IL-1β の神経保護作用のメカニズムについて、５）親電子性物質による中毒への対処法、６）誘導型のコンディショナルノックアウトマウスを使った研究の可能性ついて質疑が行われ、申請者より的確な回答が得られた。

本論文は代謝によって親電子性物質に変換されるベンゾピレンへの曝露がモノアミン作動性神経を変性させるとともに、炎症促進サイトカイン遺伝子発現を誘導すること、さらに、炎症促進サイトカインの一つである IL-1β が神経保護的に働くことを明らかにした。また、その神経保護作用はグルタチオン合成およびグルタチオンペルオキシダーゼの上方制御が関与していることを研究は示唆した。以上より本論文は博士（薬科学）の学位論文として十分に価値のあるものと認められる。