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Methamphetamine-induced dopaminergic toxicity prevented owing to the neuroprotective effects of salicylic acid

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ABSTRACT

Aims: Methamphetamine (Schedule-II drug, U.S. Drug Enforcement Administration) is one of the most abused illicit drug following cocaine, marijuana, and heroin in the USA. There are numerous health impairments and substantial economic burden caused by methamphetamine abuse. Salicylic acid, potent anti-inflammatory drug and a known neuroprotectant has shown to protect against toxicity-induced by other dopaminergic neurotoxins. Hence, in this study we investigated the neuroprotective effects of salicylic acid against methamphetamine-induced toxicity in mice.

Main methods: The current study investigated the effects of sodium salicylate and/or methamphetamine on oxidative stress, monoamine oxidase, mitochondrial complex I & IV activities using spectrophotometric and fluorimetric methods. Behavioral analysis evaluated the effect on movement disorders-induced by methamphetamine. Monoaminergic neurotransmitter levels were evaluated using high pressure liquid chromatography-electrochemical detection.

Key findings: Methamphetamine caused significant generation of reactive oxygen species and decreased complex-I activity leading to dopamine depletion. Striatal dopamine depletion led to significant behavioral changes associated with movement disorders. Sodium salicylate (50 & 100 mg/kg) significantly scavenged reactive oxygen species, blocked mitochondrial dysfunction and exhibited neuroprotection against methamphetamine-induced neurotoxicity. In addition, sodium salicylate significantly blocked methamphetamine-induced behavioral changes related to movement abnormalities.

Significance: One of the leading causative theories in nigral degeneration associated with movement disorders such as Parkinson's disease is exposure to stimulants, drugs of abuse, insecticide and pesticides. These neurotoxic substances can induce dopaminergic neuronal insult by oxidative stress, apoptosis, mitochondrial dysfunction and inflammation. Salicylic acid due to its antioxidant and anti-inflammatory effects could provide neuroprotection against the stimulants or drugs of abuse.

1. Introduction

Methamphetamine (synonym = meth, chalk, ice, crystal) is a popular drug of abuse around the world that primarily affects the central nervous system and the cardiovascular system [29]. Methamphetamine abuse has enormously increased leading to enhanced emergency department visit resulting in huge medical expenditure. However, the major concern is the long-lasting health impairments that are associated with the chronic use of stimulants. Similarly, methamphetamine use leads to addiction as evident by compulsive drug use which is associated with eminent behavioral changes, additional functional and molecular alterations of the neuron and glial cells. Due to the biochemical and neurochemical changes, it can result in movement, mental and memory related functions. Researchers have examined the role of neuroinflammation, mitochondrial dysfunction and oxidative stress associated with the common substances of abuse and illegal street drugs [13]. The substantia nigra pars compacta region of the midbrain, which is mainly composed of neuromelanin pigmented dopaminergic neurons, controls the involuntary movement related functions of the body. It also has the highest density of microglia and iron in the brain. Postmortem investigation of Parkinson's and Alzheimer's disease patients exhibits prominent reactive gliosis, augmented oxidative stress and mitochondrial dysfunction which substantiate the role of mitochondria, oxidative stress and inflammation in neurodegeneration [2,3,14,16,27].

Methamphetamine displaces dopamine from the monoaminergic storage vesicles in the pre-synaptic neurons resulting in large amounts of monoamines released into the synaptic cleft and cytosol. Once released, the dopamine owing to its high chemical instability can be oxidized to highly reactive quinone adducts, which further augments reactive oxygen species levels. These reactive species can cause

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inhibition of mitochondrial ATP production that eventually leads to depolarized mitochondrial membrane potential and mitochondrial dysfunction. The combination of oxidative stress and mitochondrial dysfunction creates degeneration of the dopaminergic neurons in the nigrostriatal nerve terminals [26,36]. The central dopaminergic system that plays such an important role in the control of motor activity is comprised of a surprisingly small number of neurons. Due to this, it is especially vulnerable and even minor insults may lead to irreparable behavioral and biochemical deficits [7].

Thus, a drug that could provide neuroprotection against oxidative stress, mitochondrial abnormality and inflammation can prevent neuronal neurodegeneration associated with substances of abuse and street drugs like methamphetamine and its structural congeners.

Salicylic acid pharmacologically is characterized as analgesics or antipyretics [11]. Salicylic acid is a metabolite produced in the body following ingestion of acetylsalicylic acid (aspirin). Aspirin was the first-discovered member of the non-steroidal anti-inflammatory drugs (NSAIDs), which all have similar effects and most have inhibition of the cyclooxygenase as their principal mechanism of action. Today, aspirin is one of the most widely used medications in the world, with an estimated 40,000 metric tons of it being consumed each year. Salicylic acid is a known free radical scavenger [12] and possesses significant antioxidant properties. It has been shown to provide neuroprotection against MPTP induced neurotoxicity [17]. With the ability of salicylic acid for free radical scavenging, it could play a much needed neuroprotective role in not only nigral dopaminergic neurodegeneration, but also in numerous other neurodegenerative disorders. Our earlier studies show that administration of methamphetamine to mice produces a loss of dopaminergic neurons and a syndrome that behaviorally, biochemically, and neurochemically resembles Parkinson's disease [28]. Presently there are no specific therapeutic strategies that neutralize the explicit adverse actions of methamphetamine & its structural congeners, or therapies that prolong abstinence and decrease the abuse of methamphetamine. Hence, in this study, we use methamphetamine treated mice to establish the neuroprotective effects of salicylic acid. If salicylic acid proves to be a neuroprotectant, preventing methamphetamine-induced neurotoxicity, it could be used clinically for abuse and toxicity associated with designer drugs, substances of abuse and importantly can slow the progression of neurodegeneration.

2. Materials and methods

2.1. Animals

All the experimental procedures for this study pertaining to the neuroprotective effects of salicylic acid against methamphetamine-induced neurotoxicity were reviewed and approved by Institutional Animal Care and Use Committee (IACUC) at Auburn University. Male C57/Bl6 mice purchased from Charles Rivers were housed for 2–4 days prior to experiments in a temperature controlled room with a 12 h day and night cycle with free access to food and water. We also weighed the animals regularly to look for any changes.

2.2. Drug administration

C57/Bl6 mice were separated into 5 groups (control, methamphetamine only, high dose sodium salicylate only (100 mg/kg), sodium salicylate high dose (100 mg/kg) + methamphetamine and sodium salicylate low dose (50 mg/kg) + methamphetamine). The groups were given intraperitoneal (i.p.) injections once daily for one week with sterile water (control & methamphetamine groups), 100 mg/kg sodium salicylate (high dose), 100 mg/kg sodium salicylate (sodium salicylate high dose + methamphetamine group), and 50 mg/kg sodium salicylate (sodium salicylate low dose + methamphetamine group). On day 7 the methamphetamine, sodium salicylate high dose + methamphetamine and sodium salicylate low dose + methamphetamine groups were injected with methamphetamine (10 mg/kg i.p., twice, 2 h apart). The animals were sacrificed 5 days after the last injection.

2.3. Behavioral studies

Akinesia, Catalepsy and Swim test were performed based on previously established standard procedure [6,20,28,33].

2.4. Tissue preparation for in vivo biochemical assays

To avoid diurnal discrepancies of the changes in endogenous amines, enzymes, and other antioxidant molecules, control and drug treated mice were sacrificed by decapitation in the morning. Various brain regions were homogenized in 0.1 M phosphate buffer (pH 7.8) and centrifuged at 10,000g for 60 min at 4 °C and the supernatants were used for various assay [19,20].

2.5. Dopamine content

Striatum was dissected and analyzed for dopamine content using HPLC-electrochemical detector (HPLC-ECD) according to our previously published procedure [31].

2.6. Protein estimation

Protein was assayed using the coomassie plus protein assay reagent kit (Pierce, Rockford, IL). Bovine serum albumin (BSA) was used as a standard for protein measurement.

2.7. Mitochondrial complex I activity

Mitochondrial complex-I activity (NADH dehydrogenase activity) is measured spectrophotometrically based on the NADH oxidation. The mitochondrial complex-I activity is expressed as the amount of NADH oxidized/min/mg protein [24,34].

2.8. Mitochondrial complex IV activity

Complex IV activity was based on the cytochrome-C oxidation. The oxidation was measured spectrophotometrically. The absorbance was measured at 550 nm for 2 min and the enzyme activity was expressed as cytochrome-C oxidized/mg protein [24,34].

2.9. Mitochondrial monoamine oxidase (MAO) activity

Total monoamine oxidase activity was based on the amount of 4-hydroxyquinoline formed by the oxidation of kynuramine [18]. 4-hydroxyquinoline was measured fluorimetrically and the enzyme activity was expressed as 4-hyroxyquinoline formed/h/mg protein [19,20].

2.10. Assay of ROS production

The generation of ROS was measured and reported as relative fluorescence intensity [4]. Conversion of nonfluorescent chloromethyl-DCF-DA (2′,7′-dichlorofluorescin diacetate) to fluorescent DCF was used to monitor ROS production spectrofluorometerically using an excitation wavelength of 492 nm and an emission wavelength of 527 nm.

2.10.1. Super oxide dismutase (SOD) activity

Super oxide dismutase (SOD) activity was measured spectrophotometrically by Marklund and Marklund method [15] using pyrogallol as substrate at 420 nm.