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Effects of imperatorin on nicotine-induced anxiety- and memory-related responses and oxidative stress in mice

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ABSTRACT

The purpose of the reported experiments was to examine the effects of imperatorin [9-[(3-methylbut-2-en-1-yl)oxy]-7H-furo[3,2-g]chromen-7-one] on anxiety and memory-related responses induced by nicotine in mice and their relation to the level of nicotine-induced oxidative stress in brain as well as in the hippocampus and the prefrontal cortex. Male Swiss mice were tested for anxiety in the elevated plus maze test (EPM), and for cognition using passive avoidance (PA) procedures. Imperatorin, purified by high-speed counter-current chromatography from methanol extract of fruits of *Angelica officinalis*, acutely administered at the doses of 10 and 20 mg/kg impaired the anxiogenic effect of nicotine (0.1 mg/kg, s.c.). Furthermore, acute injections of subthreshold dose of imperatorin (1 mg/kg, i.p.) improved processes of memory acquisition when co-administered with nicotine used at non-active dose of 0.05 mg/kg, s.c. Additionally, repeated administration of imperatorin (1 mg/kg, i.p., twice daily, for 6 days) improved different stages of memory processes (both acquisition and consolidation) when injected in combination with non-active dose of nicotine (0.05 mg/kg, s.c.) in the PA task. Oxidative stress was assessed by determination of antioxidant enzymes (glutathione peroxidases (GPx), superoxide dismutase (SOD), glutathione reductase (GR)) activities as well as of malondialdehyde (MDA) concentration in the whole brain, the hippocampus and the prefrontal cortex after repeated administration of imperatorin (1 mg/kg, 6 days) and single nicotine injection (0.05 mg/kg s.c.) on the seventh day. The results of our research suggest strong behavioural interaction between imperatorin and nicotine at the level of anxiety- and cognitive-like processes. Furthermore, imperatorin inhibited nicotine-induced changes in examined indicators of oxidative stress, especially in the hippocampus and the cortex.

1. Introduction

Cigarette smoking is the leading cause of preventable illness and premature mortality in developed countries [1]. There is ample evidence that nicotine, as the major addictive component of tobacco, leads to abuse despite the harmful consequences [2]. Additionally, quitting smoking is highly difficult. It is estimated that about 80% of the attempts fail within a year [3]. Moreover, nicotine induces diverse behavioural effects, including the cognitive effects [4–8], anxiety-like behaviour [9], analgesia [10], or depressive-like behaviours [11]. This alkaloid acts on the neuronal nicotinic acetylcholine receptors (nAChRs), which are highly distributed in the central nervous system (CNS), mainly at the pre-synaptic level, and promotes the release of several neurotransmitters, such as acetylcholine (ACh), dopamine, noradrenalin, serotonin (5-HT) and gamma-aminobutiric acid (GABA) [12]. The actions of nicotine have been extensively investigated not only in humans, but also in animal and variety of cell system [13–15].

Imperatorin [9-[(3-methylbut-2-en-1-yl)oxy]-7H-furo[3,2-g]chromen-7-one] is one of the bioactive furanocoumarins found in fruits of *Angelica dahurica* and *Angelica archangelica* (Apiaceae) [16]. Imperatorin has also been found in popular culinary herbs, such as parsnip, parsley and fennel [17]. This compound may have potent anticarcinogenic, antihypertensive, antiproliferative, anticonvusculant and anti-inflammatory properties [18–22]. Assembled experimental evidence indicates that imperatorin's mechanism of action is connected with the voltage-dependent calcium channel and receptor-mediated calcium influxes and release inhibition, but also with competitive antagonist properties of 5-HT receptors [23], exhibiting stronger affinity towards the 5-HT_7 receptor [24]. However, in the context of presented studies, other mechanisms of action of this furanocumarin seem to be important. For instance, literature data have revealed that this compound inactivates GABA transaminase [25]. Imperatorin was also shown to inhibit the activity of acetylcholinesterase (AChE), which is an enzyme

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responsible for degradation of ACh, the neurotransmitter essential for cognitive function. Moreover, coumarins as well as their derivatives were found to exert strong antioxidant and anti-inflammatory effects [26–28], that also may influence the effects of imperatorin. These compounds were stated to affect formation and scavenging of reactive oxygen species (ROS) and to influence processes involving free radical-mediated injury in a similar way to flavonoids [26]. Many coumarins have been recognized as lipoxygenase and cyclooxygenase inhibitors [27], which makes them able to arrest or to slow down pathological processes caused by increased activities of these enzymes, such as production of neuroinflammation involving vasodilation and vasoconstriction, platelet aggregation, leukocyte chemotaxis and release of cytokines and oxidative stress.

The formation of ROS and other free radicals during metabolism is an important and normal process that ideally is compensated by an elaborate endogenous antioxidant system. However, excessive radical production and their accumulation result in oxidative stress, a deleterious process that can be an important mediator of cell damage structures, including lipids and membranes, proteins and DNA. Overproduction of ROS is associated with numerous diseases (e.g. cancer, cardiovascular disease, atherosclerosis, hypertension, etc.) also including neurodegenerative diseases (Alzheimer's disease and Parkinson's disease) [29]. Nicotine was reported to induce oxidative stress in vitro and in vivo [30,31] expressed as decrease in antioxidant enzymes activity, changes in low-molecular-weight antioxidant concentrations and increased lipid peroxidation.

The CNS is very susceptible to oxidative stress as the brain uses about 20% of whole-body consumption of oxygen and it exhibits relatively poor antioxidant defense systems [32,33]. Additionally, the brain contains large amounts of free-radical generating iron and substances like ascorbate, glutamate and unsaturated fatty acids that easily undergo redox-reaction leading to radical formation [34,35]. Antioxidant barrier to oxidative stressors consists of two systems: enzymatic and non-enzymatic ones. Enzymatic antioxidant barrier is formed by enzymes, which cooperate with each other, to inhibit excessive production of ROS and to protect against their harmful action. The most important ones are catalase [36], glutathione peroxidases (GPx) [37], superoxide dismutase (SOD) [38,39] and glutathione reductase (GR) [40]. Low-molecular-weight radical scavengers, constituting non-enzymatic antioxidant system, react with free radicals directly and detoxify them by removing their radical character throughout electron donation. That type of antioxidants is a product of metabolic processes (endogenous antioxidants) as well as some of exogenous substances provided with food. Some examples of these substances are: ascorbate, tocopherols or reduced glutathione (GSH) [29]. Malondialdehyde (MDA), the breakdown product of the major chain reactions of polyunsaturated fatty acids oxidation, is considered to be a reliable marker of lipid peroxidation-mediated oxidative stress in tissues [41].

The purpose of the present study was to determine the effects of imperatorin on anxiety and memory behaviours induced by nicotine as well as on chosen elements of antioxidant barrier in brain. Imperatorin was purified by high-speed counter-current chromatography from methanol extract of fruits of *Angelica officinalis*. We used the elevated plus maze (EPM), a standard behavioural model that can assess nicotine-induced anxiety responses. We also examined memory retention using the passive avoidance (PA) task. To evaluate possible protective effect of imperatorin on nicotine-induced oxidative stress activities of antioxidant enzymes (SOD, GPx, GR) as well as concentration of MDA were determined.

2. Materials and methods

2.1. Animals

The experiments were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warsaw, Poland) weighing 20–25 g at the beginning of the experiments. The animals were maintained under standard laboratory conditions (12 h light/dark cycle, room temperature 21 ± 1 °C) with free access to tap water and laboratory chow (Bacutil, Motycz, Poland) and were adapted to the laboratory conditions for at least one week. Each experimental group consisted of 7–15 animals. All experiments were conducted according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), and were approved by the local ethics committee. The different mice were used for each drug and time treatment.

2.2. Drugs

The following compounds were tested: (–) nicotine hydrogen tartrate (0.05 and 0.1 mg/kg, Sigma–Aldrich, St. Louis, MO, USA) and imperatorin (8-isopentenyloxypsoralen [9-[(3-methylbut-2-en-1-yl) oxy]-7H-furo[3,2-g]chromen-7-one] (1, 10 and 20 mg/kg). Imperatorin was extracted from fruits of *Angelica officinalis* (*Angelica archangelica*, Apiaceae) collected in August 2011 in Medicinal Plant Garden, Medical University in Lublin, Poland. The air-dried and powdered fruits were extracted under reflux with methanol. The extract was separated in high-seed counter-current chromatograph Spectrum (Dynamic Extractions, Slough, UK) equipped with semipreparative coil with 137 ml capacity. System composed of heptane – ethyl acetate – methanol and water 1 : 1 : 1 : 1 was used for separation, the upper phase was used as a stationary phase. The apparatus was rotated at 1600 rpm, the mobile phase was pumped into the column at a flow rate 6.0 ml/min and the effluent from the coil was monitored at 254 nm.

The identity and purity of imperatorin were confirmed by HPLC and ^{1}H NMR analyses. Nicotine was dissolved in saline solution (0.9% NaCl) and administered subcutaneously (s.c.) at a volume of 10 ml/kg. The pH of the nicotine solution was adjusted to 7.0. Imperatorin was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (i.p.) at a volume of 10 ml/kg. Fresh drug solutions were prepared on each day of experimentation. Control groups received saline injections of the same volume and via the same route of administration.

2.3. The EPM procedure

The experimental apparatus was shaped like a “plus” sign and consisted of a central platform (5 × 5 cm), two open arms (30 × 5 cm) opposite to each other and two equal-sized enclosed (30 × 5 × 15 cm) arms opposite to each other. The maze was made of dark Plexiglas, elevated to a height of 50 cm above the floor and illuminated by dim light.

The used procedure was chosen based on our recently published data [42]. The procedure was similar to the method of Lister [43]. The test consisted of placing a mouse on the central platform facing an enclosed arm and allowing the animal to explore the maze freely for 5 min. The entry into one arm was defined as the stage when the animal placed all its four paws past the line, which divided the central square from the open arms. The test arena was wiped with a damp cloth after each trial. The number of entries into the enclosed arms and also the time spent in the open arms were measured by an observer blind to drug treatment. Anxiolytic activity was indicated by an increase of the time spent in the open arms or in the number of entries to the open arms; anxiogenic effects were characterized by a decrease in those measures. The percentage of time spent on the open arms was calculated, just as was the percentage of entries into the open arm. Additionally, the number of enclosed arm entries was recorded as the indicator of motor activity of tested animals.

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