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Original article

Potential pro-oxidative effects of single dose of mephedrone in vital organs of mice

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ABSTRACT

Background: Mephedrone is a recreationally used synthetic cathinone, relatively new abusive substances with molecular structure similar to amphetamine. As there is still lack of scientific data regarding mechanisms of action as well as metabolism of mephedrone, especially in aspects other than neurotoxicity, addiction or behavioral changes, therefore we aimed, for the first time, to investigate potential pro-oxidative actions of a single dose of mephedrone in organs other than brain and its structures, i.e. in liver, kidneys, heart and spleen of Swiss mice.
Methods: The following biomarkers of oxidative stress were measured: concentration of ascorbic acid (AA) and malondialdehyde (MDA) as well as total antioxidant capacity (TAC) of the tissues homogenates.
Results: Our study revealed that mephedrone intoxication induces oxidative stress by reducing concentration of AA and TAC and increasing concentration of MDA in these organs.
Conclusions: Such occurred state of antioxidant-oxidant imbalance may be etiopathological factor of a number of severe diseases within cardiovascular, digestive as well as immunological systems.

Introduction

Mephedrone (MEPH, 4-methylmethcathinone, 4-MMC), an illegal in the USA and many EU countries [1,2], synthetic cathinone, recreationally used stimulating drug [3] was first time synthesized in 1929 but has not become widely known and used until 2000's. It is available in the form of tablets, capsules and powder, which can be dissolved and injected [2,4,5]. It has become a cheaper alternative to amphetamine for the reason that it is easier to produce [6]. Mephedrone is sold under many street names [2,7] while one of most popular is "bath salt". Some studies found it as the mixture of mephedrone and 3,4- methylenedioxypyrovalerone (MDPV) – other synthetic cathinone [8]. Growing popularity of mephedrone among drugs users could be stated throughout more and more numerous cases of MEPH intoxication that have been described; moreover few fatal cases have been also reported [9–11].

There is a relative paucity of scientific data regarding mechanisms of action as well as metabolism of mephedrone, especially in aspects other than neurotoxicity, addiction or behavioral changes. Some case reports are available, although they contain a medical description of club drugs poisoning and there is not 100% certainty which drug they refer to. Therefore, doctors in toxicological departments still do not have proven knowledge regarding treatment and detoxification specific to mephedrone intoxication. Thus, research on the influence of mephedrone on living organisms in all aspects appears to be direly required.

We chose liver, heart, kidneys and spleen for this study as they have fundamental functions for living organisms. Any dysfunction of these biological structures may lead to multi organ failure or directly to death. Moreover, these organs are crucial in defending the individual against poisoning toxins. Liver is responsible for detoxification, kidneys remove toxins from the body and spleen plays a crucial role in proper functioning of the immunological system. What is more, there are no studies on mephedrone-induced oxidative stress on these systems. The idea of investigating pro-oxidative effects of mephedrone in peripheral organs seemed very important in terms of toxicological aspects.

As mechanisms of mephedrone toxicity have not been well known yet, noteworthy is the fact that mephedrone is a compound from a group of β-ketone amphetamines. Therefore, its structural similarity to amphetamine and its derivatives (like methylenedioxymethamphetamine, MDMA), as well as the similarity of these compounds in terms of properties, toxicological profile and

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metabolic pathways could be assumed. And so, amphetamine, as well as MDMA, have proven pro-oxidative properties [12,13], which means that they generate excessive amounts of reactive oxygen species (ROS).

ROS are free radicals with the unpaired electron and for that reason, they are highly reactive and have a very short lifetime in normal conditions. ROS are physiology created during electron respiratory chain metabolism and serve as cellular signaling molecules as well as cellular response to the bacterial invasion and proinflammatory cytokines. ROS are also proven to be important factors in aging and pathological processes like carcinogenesis and neurodegeneration [14]. Due to the fact that the oxidizing agents can damage all cell components at the molecular level, the cells have different ways to protect themselves against the harmful effects of oxidants. The antioxidant barrier consists of two main systems of protecting components of the cell against ROS: antioxidant enzymes like: superoxide dismutase (SOD) [15], catalase (CAT), glutathione peroxidase (GPx), glutathione reductase[16] and low-molecular-weight antioxidant (LMWAs) – a group of substances capable of preventing damage by direct interaction with ROS [17].

In all living cells, ROS are constantly created and neutralized by LMWA and antioxidant enzymes. Both of these processes are usually in a state of a balance. However, the excessive production of ROS may overcome the antioxidant barrier capability and cause oxidative stress, which is the state of imbalance between oxidants and antioxidants. LMWAs are used in the process of neutralizing ROS.[17] If they are not resynthesized by the cell, they will be used completely and the cell will lose antioxidant defending mechanisms. In such situation, ROS may significantly damage nuclear and mitochondrial DNA, important proteins and lipids of cell's membrane.[18]

ROS may interact with the cell in two main ways – decrease antioxidant capacity or/and cause damage to its structure. In order to investigate both mechanisms, the following biomarkers of oxidative stress were chosen: (1) concentration of reduced ascorbic acid (AA), which is one of the LMWAs. Mice cells are able to synthesize AA themselves in contrary to human ones [19] so for that reason, organs were collected shortly after drug injection. (2) Total antioxidant capacity (TAC) describes the general ability of cell to protect itself against oxidative stress. This reflects concentration of LMWA and antioxidant activity of the enzymes. (3) The concentration of malondialdehyde (MDA), the main product of lipids oxidation, reflects damages done by ROS to lipids of the cells, which are mainly present in the cell membrane.

Taking into consideration pro-oxidative effects of amphetamine and its derivatives as well as structural similarity of mephedrone to amphetamines, we aimed to investigate, for the first time, possible the pro-oxidative actions of a single dose of mephedrone in organs (liver, kidneys, heart and spleen) of Swiss mice.

Materials and methods

Ethics statement

The study was carried out according to the National Institute of Health Guidelines for Care and Use of Laboratory Animals and the European Community Council Directive for the Care and Use of Laboratory Animals of 24 Nov 1986 (86/609/EEC) and was approved by the local ethics committee (Permit Number: 48/2013). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animals

The experiments were performed on 2-month old naïve Swiss male mice, weighing 20–25 g at the beginning of the experiments. The mice were kept under standard laboratory conditions (12 h light/dark cycle, room temperature 21 ± 1 °C) with free access to tap water and laboratory chow. Each experimental group consisted of 10 mice. The animals had been adapted to the laboratory conditions for at least 1 week.

Drug

In the experiment mephedrone: [(RS)-2-methylamino-1-(4-methylphenyl] propan-1-one (Toronto Research Chemicals Inc.) was tested. The drug was dissolved in saline solution (0.9% NaCl) and administered intraperitoneally (*ip*) at the doses of 1, 2.5, 5 and 10 mg/kg. The volume of injections was 10 ml/kg b.w. The control groups received saline injections of the same volume and *via* the same route of administration. The doses of mephedrone were chosen based on literature data [6,20–22], our recently published articles [23] and preliminary studies.

Experimental protocol

The mice were randomly divided into 5 groups with 10 animals each and treated *ip* as follows: (1) the control group (saline), (2) mephedrone 1.0 mg/kg, (3) mephedrone 2.5 mg/kg, (4) mephedrone 5.0 mg/kg, (5) mephedrone 10.0 mg/kg b.w. Animals were decapitated one hour after the injection. The tissue (liver, kidneys, heart and spleen) were removed and rinsed with ice-cold saline to remove blood and then stored at −20 °C for further analysis. Then, the collected tissues were homogenized on ice in 10 volumes of cold 20 mM Tris-HCl buffer (pH 7.4) and centrifuged at 4 °C at 10,000 rpm for 10 min to separate nuclear debris. The supernatant was collected and used for the study.

Biochemical procedures

Oxidative stress was evaluated by measurements of the TAC, the concentration of reduced AA and the total amount of lipid peroxidation products determined by the concentration of MDA in the tissues homogenates.

Measurement of TAC

The determination of TAC of tissue homogenates was determined by ferric-reducing ability of plasma (FRAP) method with modifications of Benzie and Strain for tissue homogenates supernatants and adapted to microplate assays [24]. The method is based on the evaluation of the antioxidant capacity of a tissue by estimating the concentration of all substances able to reduce ferric ions. Briefly, working reagent was prepared by mixing acetate buffer of pH 3.6 with 2,4,6-tri-pyridyl-s-triazine (10 mM) in 40 mM HCl and the aqueous solution of $FeCl_3$ (20 mM) in the ratio of 10:1:1. The standard curve was prepared with $FeSO_4$ at concentrations from 0 to 1000 μM. Then, 10 μl of standards and samples were mixed with 20 μl of deionized water and 200 μl of working reagent at 96-well plate. The course of reaction of Fe(III)–tripyridyltriazine (Fe(III)–TPTZ) reduction into to blue Fe(II)–tripyridyltriazine (Fe(II)–TPTZ) was measured spectrophotometrically at 593 nm after 30 min of incubation at 37 °C. The results evaluated from the standard curve were recalculated per protein content of supernatants and expressed as micromoles per gram of protein.

Determination of AA concentration

The concentration of AA was determined using modified Kyaw method [25]. Briefly, 1 ml of analytical reagent containing phosphotungstic acid was added to 1 ml of tissue homogenate supernatant. After centrifugation, the absorbance of samples was read at 700 nm on HITACHI 20800 spectrophotometer and the