Ellagic acid, a polyphenolic compound, selectively induces ROS-mediated apoptosis in cancerous B-lymphocytes of CLL patients by directly targeting mitochondria

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ABSTRACT

To investigate the effects of ellagic acid (EA) on the cytotoxicity, B-lymphocytes from CLL patients and healthy individuals. Flow cytometric assay was used to measure the percentage of apoptosis versus necrosis, intracellular active oxygen radicals (ROS), mitochondrial membrane potential (MMP) and the caspase-3 activity and then mitochondria were isolated from both groups B-lymphocytes and parameters of mitochondria. It was investigated. Based on our results EA decreased the percentage of viable cells and induced apoptosis. EA increased ROS formation, mitochondria swelling, MMP decrease and cytochrome c release in mitochondria isolated from CLL BUT NOT healthy B-lymphocytes while pre-treatment with cyclosporine A and Butylated hydroxytoluene (BHT) prevented these effects. Our results suggest that EA can act as an anti-cancer drug and could induce apoptosis through mitochondria pathway with increasing ROS production which finally ends in cytochrome c release, caspase-3 activation and apoptosis in cancerous B-lymphocytes isolated from CLL patients.

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1. Introduction

Cancer is a leading cause of death in developed and developing countries [1,2]. Searching for new anti-cancer agents from plant and other sources is a promising approach which may lead to the discovery of many novel anti-cancer agents [3,4]. Chronic lymphocytic leukemia (CLL) is a neoplasm characterized by clonal proliferation and accumulation of mature B-lymphocytes in the blood, lymph nodes, spleen and bone marrow. CLL is the most common leukemia in Western countries, and it accounts for approximately one-third of all leukemias in the United States and other countries [5]. The median age for diagnosis of CLL is 65 years, and the disorder is higher in men than women [6]. However, despite the initial effectiveness of used anti-cancer drugs in patients with low-grade disease, resistant cells ultimately emerge, leaving no effective treatment options available. It is possible that drug-resistant CLL cells possess intrinsic defects in their ability to undergo apoptosis. Apoptosis is a physiological cell suicide program that is essential for the regulation of development, the maintenance of homeostasis and the prevention of tumorigenesis [7]. Evading the apoptotic program is one of the hallmarks of cancer and represents an important mechanism in clinical re- sistance to cancer therapies [8]. This is particularly true for chronic lymphocytic leukemia (CLL), a currently incurable condition that is clearly characterized by impaired apoptosis [10]. The development of therapeutic strategies that target apoptosis in CLL is therefore a very important issue [11]. CLL is typically sensitive to a variety of cytotoxic drugs, but the disease is considered incurable [12]. Treatment is generally recommended to control symptoms and reduce bulk of disease but without substantially improving survival [13]. New targets such as mitochondria has facilitated the development of new drugs with a view to improving clinical outcomes for this neoplasm [14]. It is now clear that mitochondria are central to cell death, cell differentiation, innate immune system, hypoxia sensing, metabolism of calcium and amino acids, iron sulfur center and heme biosynthesis [15]. Disruption to these processes contributes to several pathologies, such as cancer, making mitochondria an important therapeutic target [16]. In
Selective Anticancer Activity of Acacetin against Chronic Lymphocytic Leukemia Using both in Vivo and in Vitro Methods: Key Role of Oxidative Stress and Cancersous Mitochondria

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is a malignancy with limited therapeutic options. The present study investigates the in vitro and in vivo effect of acacetin (4'-methoxy-5,7-dihydroxy flavone) on CLL B-lymphocytes and mitochondria. CLL B-lymphocytes and healthy B-lymphocytes were obtained from CLL patients and healthy donors, respectively. Mitochondria were isolated from B-lymphocytes of both groups. Xenografts in severe combined immune deficient mice were used to examine the toxicity and anti-CLL activity of acacetin. We evaluated and compared the mechanism of action of acacetin on CLL and healthy B-lymphocytes and their mitochondria. We have found that acacetin (10 μM) can selectively induce apoptosis on CLL B-lymphocyte (% 25 at 24 h) by directly targeting mitochondria, through increased reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP) collapse, mitochondrial permeability transition (MPT), release of cytochrome c, caspase 3 activation, and finally apoptosis, while sparing normal healthy B-lymphocytes unaffected at similar concentrations. Besides, acacetin oral administration showed a potent in vivo anticancer activity in CLL xenograft mouse models. Our in vivo findings indicate that acacetin accumulates and kills CLL B-lymphocyte in a rather selective way through targeting cancersous mitochondria and ROS formation which ends in CLL therapy. Finally, we can recommend acacetin as a promising compound for further drug development assays for the CLL treatment.

Keywords: Apoptosis, Flavonoids, Oxidative stress, Tumor models

Introduction

Chronic lymphocytic leukemia (CLL) is one of these cancers that characterized by the relentless accumulation of CD5+ B-lymphocytes. The CLL is very heterogeneous that some patients may live for many years, while others may rapidly die of progressive and chemotherapy-resistant conditions. There is no effective cure for CLL, and thus novel therapies have been urgently demanded for these patients with poor prognosis (1).

To discover novel anti-cancer drug candidates, there are different methodologies. One of these methodologies is capability of induction of mitochondria-mediated apoptosis by various compounds. Apoptosis or programmed cell death is a unique type of cell death observed in both physiological and pathological conditions (2). It is well established that receptor and
Standardized Extract of the Persian Gulf Sponge, Axinella Sinoxea Selectively Induces Apoptosis through Mitochondria in Human Chronic Lymphocytic Leukemia Cells

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Abstract: Sponges are important components of the Persian Gulf animal communities. The marine sponges of the genus Axinella sinoxea are a genus of sponges in the family Axinellidae. Species of Axinella sinoxea occur in the Indian Pacific Oceans and also Persian Gulf. Chronic lymphocytic leukemia (CLL) is a disease characterized by the relentless accumulation of CD5+ B lymphocytes. CLL is the most common leukemia in adults, about 25–30% of all leukemias. In this study B lymphocytes mitochondria (both cancerous and non-cancerous) were isolated using differential centrifugation from peripheral blood samples and succinate dehydrogenase activity, mitochondrial reactive oxygen species (ROS) level and the MMP of mitochondria were measured. Moreover, the release of cytochrome c from mitochondria was measured using an ELISA kit. 

Keywords: Sponge, Axinella sinoxea; Mitochondria, Chronic Lymphocytic Leukemia.

1. INTRODUCTION

Marine organisms have shown to be potential sources of bioactive compounds with pharmacological effect [1]. With bryozoans, loricates and sponges being the most promising marine organisms as sources of new active compounds for drug development [2]. Because of their presence, ease of collection, ability to biosynthesize an array of structurally diverse natural products, marine sponges have been the primary source of biologically active marine natural products [3]. The initial study on the sponge nucleosides spongouridine and spongouridine in the marine sponge Cryptotheca crypta played a major role in promoting the search for marine bioactive molecules [4]. In the recent years, marine natural products bioprospecting has yielded a considerable number of drug candidates, most still being in preclinical or early clinical development, with only a limited number already in the market [5]. Among these, several anticancer agents derived from marine sources especially sponges have entered preclinical and clinical [6-8]. These compounds (Cytarosine, Halichondrin B, Bryostatin 1, Dolastatin 10 and Ecteinascidin 743) have shown cytotoxic activity against various tumor types (leukemia and lymphoma) [9]. Cancer, as one of the most important diseases in humans, has always attracted the scientific and commercial communities with an effort of continuously discovering new anticancer agents from natural products sources [10]. Chronic lymphocytic leukemia (CLL) is a low-grade B-cell malignancy [11] identified by the gradual accumulation of a monoclonal population of CD5+CD19+ B lymphocytes [12]. Clinically, the CLL is

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Editorial

Isolated Human Peripheral Blood Mononuclear Cell (PBMC), a Cost Effective Tool for Predicting Immunosuppressive Effects of Drugs and Xenobiotics

Jalal Pourahmad and Ahmad Salimi

The science of immunotoxicology began in the early 1970s, following the recognition of increased sensitivity to infection following exposure of test species, including monkeys, hamsters, ducks, rats, mice and guinea pigs to various xenobiotics. Reduced resistance to infectious disease was a well-documented consequence of primary and acquired immunodeficiencies, but a novel outcome of xenobiotic exposure, leading some to characterize xenobiotic-induced immunosuppression as “chemical AIDS”. Although the comparison was scientifically inappropriate, “immunotoxicity” was often thought of as synonymous with “immunosuppression” during the formative years of the discipline, although hypersensitivity, allergy, and autoimmunity were recognized as potential exposure outcomes.

Assessment of potential adverse effects on the immune system is an important component of the overall evaluation of toxicity of personal care products, chemicals and drugs. Current public opinion and ethical considerations have stimulated efforts to reduce the number of animals used to test the toxicity of chemicals, drugs and personal care products. However, only limited effort has gone into developing in-vitro or in silico methods to detect immune dysfunction. This may be at least partially attributable to the sheer complexity of the immune response, although there has been sufficient progress to warrant continued investigation along these lines.

A peripheral blood mononuclear cell (PBMC) is any blood cell having a round nucleus such as lymphocyte, monocyte or a macrophage. These blood cells are a critical component in the immune system to fight infection and adapt to intruders. These cells can be extracted from whole blood using ficoll, a hydrophilic polysaccharide that separates layers of blood, followed by gradient centrifugation, which will separate the blood into a top layer of plasma, followed by a lower layer of PBMCs and then a fraction of polymorphonuclear cells (such as neutrophils and eosinophils) and finally a bottom layer of erythrocytes. The polymorphonuclear cells can be further isolated by lysing the red blood cells. PBMCs are widely used in research and toxicology applications.

Peripheral blood mononuclear cells (PBMC) give selective responses to the immune system and are the major cells in the human body immunity. They contain several types of cells such as lymphocytes, monocytes or macrophages.

Because peripheral blood is the place where exposure to chemicals occurs, these fundamentally important PBMCs are prone to be influenced by drugs and chemicals. This is why the availability of PBMCs from peripheral bloods very important for researchers studying toxicity of new drugs or drugs or drugs or chemical compounds. There are many Applications for PBMCs in toxicology research including:

1) New compound toxicity assessment; this is probably the number one reason why researchers
Toxicity of methyl tertiary-butyl ether on human blood lymphocytes

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Abstract Methyl tertiary-butyl ether (MTBE) is a synthetic solvent widely used as oxygenate in unleaded gasoline. Few studies have addressed the cellular toxicity of MTBE on some cell lines, and so far, no comprehensive study has been conducted to investigate the probable immunotoxicity of this compound. In this study, the toxicity of MTBE on human blood lymphocytes was evaluated. Blood lymphocytes were isolated from healthy male volunteers’ blood, using Ficoll polysaccharide followed by gradient centrifugation. Cell viability, reactive oxygen species (ROS) formation, lipid peroxidation, glutathione levels, and damage to mitochondria and lysosomes were determined in blood lymphocytes after 6-h incubation with different concentrations of MTBE (0.1, 0.5, 1, and 2 mM). Our results showed that MTBE, in particular, decreased cell viability, which was associated with significant increase at intracellular ROS level and toxic alterations in mitochondria and lysosomes in human blood lymphocytes. Moreover, it was shown that MTBE strongly provoked lipid peroxidation and also depleted glutathione level at higher concentrations. Interestingly, MTBE exhibited its cytotoxic effects at low concentrations that may resemble to its concentrations in human blood following occupational and environmental exposure. It is therefore concluded that MTBE was capable of inducing oxidative stress and damage to mitochondria and lysosomes in human lymphocytes at concentrations ranging from 5 to 40 μg/L, which may be present in human blood as a result of environmental exposure.

Keywords Methyl tertiary-butyl ether · Mitochondria · Oxidative stress · Cytotoxicity · Glutathione · Lysosomes

Introduction Methyl tertiary-butyl ether (MTBE) belongs to a large family of oxygenated ethers such as methyl tertiary amyl ether and ethyl tertiary butyl that came into use as motor vehicle fuel additives in the late 1970s with a significant increase in the 1990s (Scambato et al. 2009). MTBE is used as an additive to oxygenate gasoline to improve air quality by reducing tailpipe emissions of ozone precursors and carbon monoxide. Due to its enormous production, widespread use, and improper disposal of used motor oil, MTBE has been found in urban storm runoff and streams, lakes, and groundwater. Then, potential for human exposure is high (Dong-Mei et al. 2009). MTBE pollution is considered as a critical environmental problem. It is often found at contaminated sites and is thought to be harmful to human health. Exposure to MTBE has been a long time concern in many occupational fields, including gasoline transport by tanker lorries, service stations, policemen, and the petroleum industry. Traffic policemen and gas station workers are two of the groups most heavily exposed to vehicle exhausts during their work shifts (Eggehy et al. 2000, Crebelli et al. 2001, Scibetta et al. 2004). According to literature, in estimation of workers’ exposure with biological samples,
Use of Nutraceuticals for Prevention and Treatment of Cancer

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Nutraceuticals, mostly phytochemicals derived from dietary or medicinal plants such as soya bean, garlic, ginger, tea as well as propolis, honey and others, may have chemopreventive activities, as already suggested by epidemiologic and animal model studies. Their ability to reduce cancer incidence in these studies is likely related to apoptosis. The potential of using nutraceuticals as chemopreventive reagents has prompted a surge of in-vitro study on their biological effects in cultured human cells. Chemoprevention is the use of small molecules, including dietary or herbal chemicals, to prevent cancers, as opposed to chemotherapeutics, where chemicals, mostly synthetic, are used to remove or alleviate cancer symptoms. The concept of chemoprevention, although prevalent in the East for thousands of years, has not gained scientific recognition in the West until recently. Large scale clinical studies have demonstrated the efficacy of using tamoxifen, raloxifene, both estrogen receptor antagonists, and fenretinide, a synthetic retinoid, in protecting women from breast cancer. The report by the Chemoprevention Working Group in the American Association for Cancer Research was a watershed that signaled the acceptance of chemoprevention as a viable alternative means in cancer control. Therefore, it is of interest to explore the possibility of using phytochemicals or other dietary chemicals as chemopreventive agents. Beside, the study of the biological effects of these phytochemicals at cellular level provides the molecular basis for their anti-tumor function and helps to establish the platform for generating more potent chemopreventive and even chemotherapeutic agents. Apoptosis is involved in a whole array of normal physiological processes, including immune defense, tissue homeostasis and development, and any tilt of the balance between life and death within an organism which can lead to absence or enhancement of diseases. Thus, the loss of essential cells of post-mitotic tissues due to enhanced cell death may play an important role in a number of functional deficiencies and degenerative diseases such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, multiple sclerosis, myocardial infarction, arteriosclerosis, chronic inflammation, rheumatoid arthritis, sterility, or cataract. However, apoptosis can be considered as a proactive self-defense mechanism of a living organism to weed out dysfunctional cells such as the precursors of metastatic cancer cells, without creating oxidative stress due to inflammation. Indeed, defect in apoptosis mechanism is recognized as an important cause of carcinogenesis. A dysregulation of proliferation alone is not sufficient for cancer formation; a suppression of apoptotic signaling is needed. Cancer cells acquire resistance to apoptosis by overexpression of antiapoptotic proteins (Bel-2, IAPs, and FLIP) and/or by the downregulation or mutation of proapoptotic proteins (Bax, Apaf-1, caspase-8, and death receptors). Overexpression of antiapoptotic Bel-2 and Bel-xL probably occurs in more than 50% of all cancers. Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure, and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavonoids, isoflavones, flavonols, flavanone, and flavononoids. Among them,
Natural Compounds Target Mitochondrial Alterations in Cancer Cell: New Avenue for Anticancer Research

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Mitochondria are semi-autonomous organelles that play essential roles in cellular metabolism and programmed cell death pathways. Functional, structural and genomic alterations in mitochondria have been associated with carcinogenesis in different cells. Some of those alterations may provide a selective advantage to cells, allowing them to survive and grow under different stresses. Due to the specific alterations that occur in cancer cell mitochondria, these organelles may provide promising targets for cancer therapy. The development of drugs that specifically target metabolic and mitochondrial alterations in tumor cells has become a matter of interest in recent years, with several molecules undergoing clinical trials. This Editorial focuses on the most relevant mitochondrial alterations found in tumor cells, for cancer therapy. Since transformed cells have metabolic and mitochondrial needs that differ from their normal counterparts, mitochondrial alterations in cancer cells can offer a window of opportunity for efficient and selective anti-cancer therapy. This principle of oncogenic alterations is in fact the basis for new anticancer research and can also be used with success when targeting altered aspects of tumor cell mitochondria. All the previous published works in this regard suggest that there is no mitochondrial alteration common to all cancer types, which excludes the use of a single mitochondrial-acting agent for all cancers. There are candidate natural compounds, in different stages of drug development, which have been described to interfere with mitochondrial functions in tumor cells, leading to cell cycle arrest and/or death by apoptosis or necrosis. Natural compounds have long been studied for their anticancer properties. Chemicals resemble ubiquinone can act to inhibit complex II via competitive binding such as vitamin E analogs. For instance, agents such as α-tocopherol succinate (α-TOS) a vitamin E analog, represent excellent candidates for cancer therapy. α-TOS competes with ubiquinone in binding to Q sites on complex II of the respiratory chain. This connection, through electron flux, destabilizes the mitochondrial membrane consequently and generates superoxide anion. Furthermore; α-TOS promotes Bax translocation to the mitochondrial outer membrane and subsequent cytochrome c release resulting in programmed cell death. Resveratrol, a natural stilbene, was shown to act as a mitoain, compounds which specifically target mitochondria in tumor cells. Resveratrol can act directly at several different sites from complexes I to III, possibly competing with ubiquinone binding sites. Moreover, it interacts with complex V, the F0–F1 ATP synthase. Although Resveratrol is generally considered a potent antioxidant, it can also act in favor of oxidative status in cells, inducing apoptosis through the mitochondrial pathway. The chemotherapeutic properties of Curcumin, the major constituent of turmeric powder, have been reported as inducing lung cancer cell death through Bax up regulation.
Mitochondrial Targeting for Drug Development

Jalal Pourahmad, Ahmad Salimi and Enayatollah Seydi

Additional information is available at the end of the chapter

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1. Introduction

What is the reason that mitochondrion is so attractive target for pharmacotherapy? The problem raised is a bit complex. Although the appearance of mitochondria in animal cells started more than one million years ago, when the proteobacteria (in particular, Rickettsiales or close relatives) entered and practiced coexistence inside eukaryotic cells via endocytosis, but the biological science started to pay attention on mitochondria in the middle of 19th century, the time that mitochondria were discovered in tissue section of liver and flight muscle. The earliest definition of this organelle in cells was written by Richard Altman on 1890, when he named them as “bioblasts” and hypothesized that they were “elementary organisms” living inside cells with “vital functions” [1]. After 1890, the progress in understanding the structure and function of mitochondria was quite steady, but we can single out major milestones in every decade. In the 1950s, investigators analyzed mitochondria by electron microscopy and characterized that they are the sites of respiration, Oxidative Phosphorylation (OXPHOS) and fatty acid oxidation. In the 1960s, investigators found out mitochondrial DNA (mtDNA) and described chemiosmotic theory. In the 1980s, the first consummate sequence of mammalian mtDNA and the first molecular identification of a cause of mitochondrial diseases were reported. A great incrementation in interest on mitochondria occurred in 1996, when researchers demonstrated that the organelles are associated with programming cell death or apoptosis [2]. After this manifestation, mitochondrial study commenced growing as a biomedical field. Owing to the unique structure and function of mitochondria, several clinically used drugs can improve or damage their bioenergetics. These drugs can act via the regulation of: (a) permeability transition pores (PTPs), (b) fatty acid uptake or oxidation, (c) the electron transport chain (ETC), (d) cardiolipin (CL) content (c) ion channels and transporters, (f) Adenosine triphosphate (ase) (ATPase) and (g) mtDNA and protein synthesis [3]. Mitochondria are subcellular organelles that play pivotal roles essential for energy (ATP) production, metabolism,
4-(4-(Methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one: a novel COX-2 inhibitor acting selectively and directly on cancerous B-lymphocyte mitochondria

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A new series of 3-phenoxyazetidin-2-ones (β-lactams) were designed and synthesized for the evaluation as selective cyclooxygenase-2 (COX-2) inhibitors. In this study, the effects of a synthetic of β-lactam-structured COX-2 inhibitor with 4-(4-((methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one on cell viability of cancerous lymphoblast isolated from patients diagnosed with acute lymphocytic leukemia (ALL) and normal lymphocytes collected from healthy donors were investigated. The viability % of cancer lymphoblast and normal lymphocyte treated-4-(4-(methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one were tested with MTT assay. Apoptosis and necrosis were measured by double stains of annexin V and propidium iodide, and caspase-3 as a final mediator in apoptotic death measured by colorimetric assay. Mitochondria were isolated from both cancerous lymphoblast and normal lymphocytes to measure parameters of mitochondrial damage such as reactive oxygen species (ROS) formation, mitochondrial membrane potential decrease, swelling, and cytochrome c release following the administration of azithidine-2-one derivative, 4-(4-(methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one. Our results showed that 4-(4-(methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one inhibited proliferation of cancerous lymphoblast in a concentration-dependent manner by inducing apoptosis but not in normal lymphocytes. Treatment with azithidine-2-one derivative produced a rapid loss of mitochondrial membrane potential, stimulation of release of ROS and mitochondrial cytochrome c into cytosol, and subsequent induction of procaspase-9 processing. Data suggest that 4-(4-(methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one-induced ROS production led to mitochondrial-mediated death signaling that resulted in apoptosis in cancerous lymphoblast cells. The induction of apoptosis by azithidine-2-one compounds, such as 4-(4-(methylsulfonyl)phenyl)-3-phenoxy-1-phenylazetidin-2-one, may provide a mechanism for its cancer chemopreventive action in acute lymphocytic leukemia cells.

Keywords: azithidine-2-one; COX-2 inhibitors; mitochondria; apoptosis; acute lymphocytic leukemia

Introduction

Acute lymphocytic leukemia (ALL) occurs in children (aged 1–15 years), accounting for almost 30% of all cancers in this age group (Pui, Relling, and Downing 2004). The disease develops at any age during childhood, but most commonly between 4 and 7 years of age (Ludwig et al. 1998; Pyatt, Aylward, and Hays 2007). Apoptosis is a unique type of
Research Article

**Dracocephalum: Novel Anticancer Plant Acting on Liver Cancer Cell Mitochondria**

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**Draecocephalum kotschyi** Boiss. (Labiateae) is a native Iranian medicinal plant which has been used in combination with *Peganum harmala* L. as a remedy for many forms of human cancer especially leukemia and gastrointestinal malignancies. Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. In this investigation HCC was induced by a single intraperitoneal injection of diethylnitosamine (DEN) in corn oil at 200 mg/kg body weight to rats. Two weeks after DEN administration, cancer development was promoted with dietary 2-acetylaminofluorene (2-AAF) (0.02%, w/w) for 2 weeks. Serum alpha-fetoprotein (AFP) concentration, serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were also determined for confirmation of hepatocellular carcinoma induction. Then rat hepatocytes were isolated with collagen perfusion technique and tumoral hepatocytes were sorted by flow cytometry. Finally isolated mitochondria obtained from both tumoral and nontumoral hepatocytes were used for any probable toxic effect of *Draecocephalum kotschyi* ethanolic extract. Our results showed that a concentration of 250 µg/mL induced reactive oxygen species (ROS) formation, mitochondrial membrane permeabilization (MMP), and mitochondrial swelling and cytochrome c release only in tumoral but not nontumoral hepatocyte. These findings propose *Draecocephalum kotschyi* as a promising candidate for future anticancer research.

1. Introduction

HCC is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism is the most common cause of hepatic cirrhosis) [1]. More than 600,000 people die from HCC each year. Worldwide research on the disease needs to be intensified in both the medical and pharmaceutical fields. The incidence of this disease is increasing and it is one of the key indications for liver transplantation. A considerable number of antitumor agents currently used in the clinic are of natural origin. Over half of all anticancer prescription drugs approved internationally are natural products or their derivatives [2, 3]. DEN is a chemical carcinogen which causes the cirrhosis in liver and other organs and is widely used to initiate hepatocarcinogenesis in rats while the further promotion of cancer phenotype might be caused by 2-AAF, phenobarbital, carbon tetrachloride, and other chemicals [4].

*D. kotschyi* is a wild-growing flowering plant belonging to the family Labiateae and is found abundantly in southwestern Asia. *D. kotschyi* has been used as a medicinal herb for several years in Iran folk medicine due to its antispasmodic and analgesic properties. Anti-hyperlipidemic [5] and immunomodulatory [6] effects have also been reported for *D. kotschyi*.

As an important organelle in the cells, mitochondria not only play a central role in calcium and energy metabolism [7], but also are essential components of the apoptotic machinery and by themselves are very important sites of reactive ROS...
Myricetin Selectively Induces Apoptosis on Cancerous Hepatocytes by Directly Targeting Their Mitochondria

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Abstract: Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death. In patients for whom HCC could not be detected early, current treatments show poor tolerance and low efficacy. So, alternative therapies with good efficacy and low toxicity are needed. The selective apoptotic effects of myricetin (MYR), a flavonoid compound, on hepatocytes and mitochondria obtained from the liver of HCC rats. In this study, HCC induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF) were used in the induction. To confirm the HCC (DEN), as an inhibitor, and 2-acetylaminofluorene (2-AAF) as a promoter. To confirm the induction, serum levels of alpha fetoprotein (AFP), AST, ALT, and ALP and histopathological changes in the liver tissue were evaluated. Rat liver hepatocytes and mitochondria for evaluation of the selective cytotoxic effects of MYR were isolated, and mitochondrial and cellular parameters related to apoptosis signaling were then determined. Our results showed that MYR was able to induce cytochrome c release only in hepatocytes from the HCC but not from rats with control diet. Besides, MYR (12.5, 25, and 50 μM) induced a considerable increase in reactive oxygen species (ROS) level, mitochondrial swelling, mitochondrial membrane permeabilization (MMP) and cytochrome c release only in cancerous hepatocytes mitochondria. MYR selectively increased caspase-3 activation and apoptotic phenotypes in HCC, but not untreated normal hepatocytes. Finally, our finding underlines MYR as a promising therapeutic candidate against HCC and recommends the compound for further studies.

Hepatocellular carcinoma (HCC) is the most common and deadliest type of liver cancer [1]. The majority of primary liver cancers (more than 90%) arises from hepatocytes and is called HCC [2]. The incidence of this disease differs widely by geographic location [3]. In contrast to other solid tumours, most HCC patients have a chronic background of liver diseases, such as hepatic fibrosis, cirrhosis, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections [4]. Several strategies such as chemotherapy and liver transplantation are used for HCC treatment. But these treatments have a limited range of applications [4]. In particular, in patients with advanced cancer could not be detected early, treatments mentioned show poor tolerance and low efficacy. So, alternative therapies with good efficacy are urgently needed [4].

Much evidence supports that intake of fruits, vegetables and other plant-derived foods containing flavonoids, a class of phenolic compounds, is frequently associated with a decreased risk of developing various cancers [5,6]. Myricetin (MYR) is a natural flavonoid commonly found in a many plants, such as tea, fruits, vegetables and medicinal plants [5,7]. Previous reports have indicated the biological activities of MYR, such as induction of apoptosis [8]. Other evidence found that the literature has focused on the pro-apoptotic properties of dietary polyphenols (such as MYR) against various types of human cancer cell lines without affecting normal cells [9,10].

In the many studies, animal models are often used to assess the anticancer effects of natural products, particularly the polyphenols [11]. Compounds such as diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF) are commonly used in cancer research to create models of cancer, especially liver cancer [12]. In this study, we aimed to determine the selective apoptotic effects of MYR on hepatocytes and mitochondria obtained from the liver of HCC-induced rats by DEN and 2-AAF in comparison with those of normal rats. So far, there are no reports on the anticaner or apoptotic effects of MYR on HCC liver hepatocytes. Our hypothesis in this current research was that MYR can selectively induce ROS-mediated apoptosis in HCC hepatocytes by directly targeting their mitochondria.

Materials and Methods

Animals. Male Sprague Dawley rats (120–130 g) fed a standard chow diet and given water ad libitum were used in all experiments. The animals for this study were purchased from Institute Pasteur (Tehran, Iran). All animals were maintained in a controlled room temperature of 20–25°C and a humidity of 50–60% and were exposed to 12-hr light/ dark cycles. The protocols approved for the study were conducted according to the ethical standards and the Committee of Animal Experimentation of Shahid Beheshti University of Medical Sciences, Tehran, Iran. All efforts were made to minimize the number of animals used and their suffering.

Experimental design. The rats were divided into two groups (5 rats in each group). A, normal rats fed with standard diet and group B (HCC group), rats were injected intraperitoneally (i.p.) with a single dose of DEN (200 mg/kg body-weight dissolved in corn oil. Two weeks after DEN administration, cancer development was promoted with dietary 2-AAF (0.02%), w/w, for 2 weeks. At the end of the HCC induction period (week 15), the body-weight of each rat was recorded and then
Selective Toxicity of Persian Gulf Sea Cucumber (Holothuria parva) and Sponge (Haliclona oculata) Methanolic Extracts on Liver Mitochondria Isolated from an Animal Model of Hepatocellular Carcinoma

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Abstract

Background: Natural products isolated from marine environments are well known for their pharmacodynamic potential in diverse disorders of the phylum Echinoderm and the class Holothuroidea, with leathery skin and gelatinous bodies. Sponges are important components of Persian Gulf animal communities, and the marine sponges of the genus Haliclona have been used for their supposed health benefits. The current study was designed to evaluate the selective toxicity of Persian Gulf sea cucumber (Holothuria parva) and sponge (Haliclona oculata) methanolic extracts on liver mitochondria isolated from an animal model of hepatocellular carcinoma, as part of a national project that hopes to identify novel potential antitumor candidates among Iranian Persian Gulf flora and fauna.

Materials and Methods: To induce hepatocarcinogenesis, rats were given diethylnitrosamine (DEN) injection (200 mg/kg, ip, by a single dose), and then the cancer was promoted with acetaminophen (APAP) (20 mg/kg, po) for two weeks. Histopathological evaluations were performed, and levels of liver injury markers and a specific liver cancer marker (alpha-fetoprotein) were determined for confirmation of hepatocellular carcinoma induction. Finally, mitochondria were isolated from cancerous and non-cancerous hepatocytes.

Results: Our results showed that H. parva methanolic extracts (250, 500, and 1000 mg/mL) and H. oculata methanolic extracts (200, 400, and 800 mg/mL) increased reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP), mitochondrial swelling, and cytochrome c release in the mitochondria obtained from cancerous hepatocytes, but not in mitochondria obtained from non-cancerous hepatocytes. These extracts also induced caspase activation, which is known as a final mediator of apoptosis, in the hepatocytes obtained only from cancerous, not non-cancerous, rats.

Conclusions: Our results suggest that H. parva and H. oculata may be promising therapeutic candidates for the treatment of HCC, following further confirmatory in vivo experiments and clinical trials.

Keywords: Carcinoma, Hepatocellular, Hepatocytes, Mitochondria, Holothuria parva, Haliclona oculata

1. Background

The liver carries out several complex and important functions, and liver diseases are considered potential threats to human life (1). Liver cancer is a common disease resulting from a several-phase process that includes the deregulation of a number of various signaling pathways (2). HCC is the most common liver cancer worldwide, the most common early cancer of hepatocytes, and the fifth most common deadly malignant tumor worldwide (3, 4). The important risk factors for HCC are environmental agents (such as hepatitis C virus, hepatitis B virus, and chemical carcinogen exposure). Several other risk factors, including food additives, non-alcoholic fatty liver disease, obesity, industrial and environmental toxic chemicals, and water and air pollutants are also involved in the etiology of HCC (5, 6). HCC is rarely detected at the primary phase, and once detected, there is a poor survival rate in most cases. HCC treatment methods, including chemotherapy, liver transplantation, and resection, show poor tolerance, low efficacy, and poor subsequent survival with a high recurrence rate (5). The diet contains several biologically active substances, nutrients, and non-nutritive compounds that may be changed into metabolites and isomers.
Toxicity of macrolide antibiotics on isolated heart mitochondria: a justification for their cardiotoxic adverse effect

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Abstract

1. Macrolides belong to the polyketal class of natural products. These products are a group of drugs (typically antibiotics) which their activity stems from the presence of a macrolide ring. Antibiotic macrolides are used to treat infections caused by gram-positive bacteria and Helicobacter pylori cause infections such as respiratory tract and cardiovascular infections. Macrolides, mainly erythromycin and clarithromycin, rarely show QT prolongation, as their infamous adverse reaction which can lead to torsades de pointes. Electrophysiological studies showed that macrolides prolonging the QT interval inhibit the rapid component of the delayed rectifier potassium current (IKr) through the block of potassium channels encoded by the human ether-a-go-go-related gene (HERG). Other studies suggest that increased Ios generation alters the kinetics of HERG K⁺ conductance.

2. In our study, rat cardiomyocytes were isolated with collagen perfusion technique. Finally, mitochondria isolated from cardiomyocytes were exposed to erythromycin, azithromycin and clarithromycin for their probable toxicity effects.

3. Our results demonstrated that macrolides induced reactive oxygen species formation, mitochondrial membrane permeabilization and mitochondrial swelling and finally, cytochrome c release in cardiomycyte mitochondria.

4. These findings suggested that the toxicity of heart mitochondria is a starting point for cardiotoxic effects of macrolides including QT prolongation, torsades de pointes and arrhythmia.

Keywords

Azithromycin, cardiotoxicity, clarithromycin, erythromycin, heart mitochondria

Introduction

Macrolides belong to one of the most commonly used families of clinically important antibiotics used to treat infections caused by bacteria such as Staphylococcus aureus, Streptococcus pneumoniae and Streptococcus pyogenes. Chemically, macrolides are represented by a 14-, 15- or 16-membered lactone ring carrying one or more sugar moieties and additional substitutions linked to various atoms of the lactone ring (Gaynor & Mankin, 2003). Erythromycin is in the macrolide class and has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often prescribed for people who have an allergy to penicillins. For respiratory tract infections, it has better coverage of atypical organisms, including mycoplasma and legionella (Camilleri et al., 2013). Gastrointestinal disturbances, such as diarrhea, nausea, abdominal pain and vomiting, are very common adverse effects of erythromycin because it is an anti-emetic agonist (Weber et al., 1993). More serious side effects include arrhythmia with prolonged QT intervals leading to torsades de pointes (Maheshwari, 2007). Clarithromycin (6-O-methyl erythromycin) is also a macrolide antibiotic used particularly for respiratory infections (Niederman et al., 2001), skin infections and Lyme disease (Wormser et al., 2006). In addition, it is used to treat Helicobacter pylori, a cause of gastric ulcer (Chew & Wong, 2007). Clarithromycin can lead to prolonged QT intervals, in patients who already have long QT syndrome, cardiodic disease or patients simultaneously taking QT-prolonging medications. This can increase risk of life-threatening arrhythmias (Hemsey & Keane, 2008). In addition, azithromycin is an azalide, a subclass of macrolide antibiotic. It is derived from erythromycin, with a methyl substituted nitrogen atom incorporated into the lactone ring, thus making the lactone ring 15-membered. Azithromycin is somewhat more potent against certain bacterial species than erythromycin (Amsden, 1996). In 2013, the FDA issued a warning that azithromycin, 'can cause abnormal changes in
Direct Toxicity of Amyloid Beta Peptide on Rat Brain Mitochondria: Preventive Role of Mangifera Indica and Juglans Regia

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Running title: Amyloid beta peptide toxicity

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Abstract

Role of mitochondrial dysfunction and oxidative stress has been well documented in various cognitive-related disorders such as Alzheimer's disease (AD). Evidence indicates that Aβ formation impairs mitochondrial function and that mitochondrial dysfunction is an early event in the pathogenesis of AD. The present study was therefore designed to investigate the direct toxicity of Aβ peptide on isolated mitochondria obtained from rat brain. Various mitochondrial toxicity/integrity parameters such as succinate dehydrogenase activity (SDH), reactive oxygen species (ROS) formation, mitochondrial membrane potential collapse (MMP), mitochondrial swelling and cytochrome c release were measured following addition of Aβ peptide on isolated mitochondria and then mitoprotective effect of aqueous extracts of Mangifera indica and Juglans regia against mitochondrial toxicity endpoints parameters induced by Aβ peptide were assessed. Our results showed that exposure to Aβ peptide (30 nM) in isolated brain mitochondria induced mitochondrial ROS formation, MMP collapse, mitochondrial swelling and cytochrome c release which is the starting point of apoptosis signaling. All these mitochondrial toxic endpoints induced by Aβ peptide inhibited by aqueous extracts of mangifera indica (100–400 μg/ml) and juglans regia (200–400 μg/ml). To our knowledge, this is one of the first apparent studies to claim directly targeting of brain mitochondria and induction of apoptosis by Aβ peptide as a new hypothesis for etiology of AD and other related neurodegenerative diseases as well as mitopreventive role of common antioxidant nutritional products including walnut and mango.

Key words: Amyloid Beta Peptide, Mitochondria, Mangifera indica, Juglans regia
Toxicity of cuprizone a Cu$^{2+}$ chelating agent on isolated mouse brain mitochondria: a justification for demyelination and subsequent behavioral and secondary dysfunction

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ABSTRACT

Multiple Sclerosis (MS) is a complex disease with an unknown etiology and no effective cure, despite decades of extensive research that led to the development of several partially effective treatments. In this study we aimed to investigate brain mitochondrial dysfunction in demyelination induced by cuprizone in mice. Cuprizone was used for induction of demyelination in mice through a diet containing 0.06% cuprizone for 5 weeks. Behavioral tests for proving of MS was performed and then mitochondria from brain of animals were isolated and afterwards parameters of mitochondrial dysfunction examined. Results of mitochondrial dysfunction, mitochondrial swelling, proton gradient, ATP production, oxygen consumption, collapse of the membrane potential showed that isolated mitochondria from cuprizone treated mice have been damaged compared to those of untreated mice. It is likely that both of unsaturated and arachidonic acid induced mitochondrial damage led to increased mitochondrial ROS formation and progression of oxidative damages in neurons. It is suggested that cuprizone which is a Cu$^{2+}$ chelating agent causes impairment of electron transport chain (complex IV) and antioxidant system (SOD) in mitochondria leading to decreased ATP production and increased ROS formation.

Introduction

Multiple sclerosis (MS) is generally accepted to be an autoimmune demyelinating disease of the central nervous system (CNS). During the 20th century, several immune-mediated animal models for MS were developed like the experimental autoimmune encephalomyelitis (EAE) model and the Thellier murine encephalomyelitis virus model. Besides these, toxin-induced demyelinating model like the cuprizone is often used to investigate the molecular factors contributing to demyelination (Ludwin, 1978). During the following three decades, cuprizone was mainly used to induce demyelination in mice (Blakemore, 1973). Moreover, cuprizone intoxication forms an excellent model to study pathology and therapy for other human diseases, including, schizophrenia and epilepsy (Bénardais et al., 2013).

Mitochondria are present in all eukaryotic cells and their prime function is to provide cells with energy, in the form of adenosine triphosphate (ATP), by oxidation of metabolic fuels. In addition, they are involved in many other important cellular processes, including fatty acid oxidation, apoptosis and calcium homeostasis (Wallace, 1992). Mitochondria also constantly produce reactive oxygen species (ROS), which have pivotal participants in cell signaling and stress responses. The high energy demands of the CNS make it particularly vulnerable to defects in mitochondrial function, as illustrated by the long list of neurological disorders caused by inherited genetic alterations in mtDNA and in nuclear genes encoding key mitochondrial proteins (Ferguson et al., 1997). Importantly, extensive mitochondrial dysfunction and concomitant oxidative stress are key features of common neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease and Huntington’s disease (Beal, 1995). Interests in mitochondria in the context of common neurodegenerative diseases were sparked. Cuprizone induces the formation of megamitochondria in liver tissue of mice. Hepatic megamitochondria in C57BL/6 mice could be observed following a 0.5% cuprizone supplemented diet, the formation of megamitochondria in oligodendrocyte was observed already after a 3 week 0.2% cuprizone diet (Acs & Komoly, 2012). Recent in vitro experiments in rat primary glial cell cultures were treated with cuprizone showed signs of toxicity including decreased survival and reduce mitochondrial transmembrane potential (Bénardais et al., 2013). In vitro, mitochondria enlarge when subjected to elevated levels of ROS and reactive nitrogen species (RNS), eventually resulting in the formation of megamitochondria (Wakehayashi, 2002). Although mitochondrial dysfunction maybe plays an important role in MS disease progression, but a better understanding of mitochondrial-related pathways is needed.

In our study, we used cuprizone for induction of demyelination in mice and then we proved symptoms disease of MS
Schizophrenia Induces Oxidative Stress and Cytochrome C Release in Isolated Rat Brain Mitochondria: a Possible Pathway for Induction of Apoptosis and Neurodegeneration

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Abstract

Schizophrenia is a chronic and often debilitating illness which affects about 1% of the world population. Some reagents have been used to simulate schizophrenic disorders in laboratory animals, such as amphetamine and ketamine. Previous studies have suggested that reactive oxygen species (ROS) production, reduced levels of ATP, mitochondrial dysfunction and apoptosis are involved in the pathophysiology and etiology of schizophrenia. In this study we divided Wistar rats into 2 groups; control group received normal saline and test group received ketamine 30 mg/Kg daily for five consecutive days. Then, locomotor activity including side to side head rocking and areing of neck, proved schizophrenia in the test group rats. Rats in both control and test groups were then decapitated and brain mitochondria were isolated. Our results showed increased ROS formation, mitochondrial membrane potential collapse, mitochondrial swelling and cytochrome c release in mitochondria of schizophrenic test group. Our findings suggested that mitochondrial signaling are likely involved in cellular pathology of Schizophrenia. To our knowledge this is the first report that provides a mechanistic justification between mitochondrial events and neurodegeneration in the Schizophrenia.

Keywords: Apoptosis; Ketamine; Mitochondria; ROS formation; Schizophrenia.

Introduction

Schizophrenia is a mental disorder characterized by a breakdown of thought processes and by a deficit of typical emotional responses (1). Prevalent symptoms include delusions and disordered thinking, as auditory hallucinations, paranoia, bizarre delusions, disordered speech, and it is accompanied by significant social or occupational imbalances. The beginning of symptoms usually happens in young adulthood, with a prevalence of about 0.3–0.7% (2). Diagnosis is based on observed behavior and the patient’s reported experiences. There are many factors that can help to this mental disorder. These factors are genetics, neurobiology, environmental, psychological and social factors (3). Some recreational and nonprescription drugs for example amphetamine, cocaine, and alcohol, can cause or worsen schizophrenia symptom (4). The main treatment for schizophrenia is antipsychotic medications, which primarily decreases dopamine receptor activity. In many cases serotonin receptor activity also suppressed. Psychotherapy and vocational and social rehabilitation also must used in treatment of schizophrenia, because these factors contribute to faster treatment. In more serious cases involuntary hospitalization may be necessary (5).
Toxicity of 4-methylimidazole on isolated brain mitochondria: using both in vivo and in vitro methods

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4-methylimidazole (4MI) is a compound widely used in various industrial and consumer applications. The most important sources of exposure include chemical caramel coloring, and pigments, dyes, cleaning and agricultural chemicals. Toxicity attributed to 4MI in foods has recently become a focus of research. Recent studies showed that 4MI induced adverse changes in various target tissues. Brain is known to be a target organ for 4MI-induced toxicity but its cytotoxic mechanisms have not yet been elucidated. In this study, experiments were divided into two parts: (1) using in vivo methodology, doses of 4MI at 100, 290, or 300 mg/kg were administered orally to mice daily for 14 to obtain brain mitochondria; and (2) utilizing in vitro methodology, brain mitochondria were incubated with 4MI at 400, 800, or 1600 μM concentrations. Subsequently, the neurotoxicity of 4MI was assessed using mitochondrial dysfunction tests, including reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP) collapse, mitochondrial swelling, and cytochrome c release. Our results from both in vivo and in vitro experiments on isolated brain mitochondria showed a significant decrease in complex II activity and also marked elevation in the ROS formation, MMP collapse, mitochondrial swelling, and enhanced release of cytochrome c. Data indicated that 4MI induced neurotoxicity through the impairment of electron transfer chain especially at complex II and elevated ROS formation leading to subsequent oxidative stress events including mitochondrial membrane depolarization, mitochondrial swelling, and release of cytochrome c, which is the starting point of mitochondrial-mediated apoptosis signaling and neurodegeneration.

Keywords: 4-methylimidazole; isolated mitochondria; in vivo; in vitro; brain

Introduction
The presence of 4-methylimidazole (4MI), a toxic compound, has raised matter concerns both in human and veterinary toxicology (Sivertsen et al. 2009). 4MI is produced by the interaction of ammonia with reducing sugars and, commercially formed by the cyclocondensation of aldehyde and ammonia with methyglyoxal (Perdok and Leng 1987). This compound may be utilized as chemical intermediate, starting material, or component in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals, and finally in the manufacture of rubber (Chan et al. 2008). 4MI has also been used as a starting material in the synthesis of epoxy resins, anti-cholesteremics, disinfectants, antiprotozoal antiseptic agents, caramel coloring, soy sauce, wine, ammoniated molasses, and caramel-colored syrups (Wong and Bernhard 1988).

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A Search for Mitochondrial Damage in Alzheimer’s Disease Using Isolated Rat Brain Mitochondria

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Abstract

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that affects regions of the brain that control cognition, memory, language, speech and awareness to one’s physical surroundings. The pathological initiation and progression of AD is highly complex and its prevalence is on the rise. In his study, Alzheimer’s disease was induced with single injection of amyloid-β (Aβ) peptides (30ng, by stereotaxic) in each hemisphere of the Wistar rat brain. Then memory dysfunction, oxidative stress and apoptosis induced by Aβ peptide were investigated in isolated brain mitochondria obtained from infected rat. Our results showed memory impairment in rats after receiving an Aβ peptide. We also found significant rise ($P<0.05$) at ROS formation, mitochondrial membrane depolarization, mitochondria swelling, cytochrome c release and significant decrease in ATP/ADP ratio on mitochondria isolated from brain of these memory impaired rats compared with those of untreated control rats group. Activation of caspase-3 the final mediator of apoptosis in the brain homogenate of the memory impaired rats was another justification for occurrence of neuron loss in the experimental model of AD. Our results suggest that oxidative stress and mitochondria mediated apoptosis in brain neurons play very important role in initiation of AD.

Keywords: Alzheimer disease; amyloid-β peptide; Mitochondria; Oxidative stress; Apoptosis.

Introduction

Alzheimer’s disease (AD) is a progressive and irreversible neurodegenerative disease described by the presence of two abnormal structures in the brain of the patients, amyloid-β (Aβ) plaques and tau neurofibrillary tangles (1-2). AD is main cause of dementia in the middle-aged and elderly (3). About 10% of the population older than 65 years of age show AD syndromes (4), and it is an important healthcare concern (2). Also this disease characterized by progressive dysfunction and decreased cognitive performance (5). In addition to the medical severity of AD, the annual cost the disease care worldwide exceeds $600 billion. At this time, more than 36 million people suffering from dementia, and this number is expected to triple by 2050 (3). In spite of study in animal models of AD, the disease remains incompletely understood, with few treatment choices (3).

Although cellular and molecular pathology and etiology of AD have not yet been completely understood, previous studies reported that mitochondrial dysfunction and oxidative stress may play roles in the development of the disease (6-7). Many studies have suggested that mitochondrial abnormalities such as increased oxygen species generation and decreased mitochondrial dynamic balance are an event in AD (1). One study reported that mutations in mitochondrial DNA (mtDNA) are worldwide for AD (8). Mitochondria are responsible for more than 90% of the cellular ATP generation (9). This bioenergetics assumes its maximum significance.

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Involvement of mitochondrial-mediated caspase-3 activation and lysosomal labilization in acrylamide-induced liver toxicity

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Acrylamide (ACR) is a chemical frequently used in both industrial and synthetic processes and may be produced during food processing. ACR at very high concentrations is postulated to exert its toxicity through the stimulation of an oxidative stress, ACR in excessive doses induces the central nervous system, reproduction, and genetic toxicity. However, ACR effects on the liver, a major organ of drug metabolism, have not been adequately explored. In addition, the role of mitochondria in an ACR-mediated hepatotoxicity is still unclear. The aim of this study was to investigate the cytotoxic mechanisms attributed to ACR using isolated rat hepatocytes. Hepatocytes were isolated by the collagenase perfusion method and incubated with an EC50m concentration of ACR for 3 hr. The EC50m of ACR on isolated rat hepatocytes was determined to be 1 mM. Based on our results, hepatocytes cytotoxicity of ACR (1 mM) was mediated by a reactive oxygen species formation and lipid peroxidation. Incubation of hepatocytes with ACR produced rapid hepatocyte glutathione depletion which is another marker of the cellular oxidative stress. ACR cytotoxicity was also associated with mitochondrial injury as evidenced by the decline of mitochondrial membrane potential and lysosomal membrane leakiness. Our results also showed that ACR induced caspase-3 activation, the final mediator of apoptosis signaling. These findings contribute to a better understanding underlying mechanisms involved in ACR hepatotoxicity originating from the oxidative stress and ending in mitochondrial/lysosomal damage and cell death signaling.

Keywords: hepatocyte; acrylamide; cytotoxicity; mitochondria; oxidative stress; lysosome

Abbreviations

ACR acrylamide
MPT pore mitochondrial permeability transition pore
GSH glutathione, reduced glutathione
GSSG glutathione disulfide, oxidized glutathione
ROS reactive oxygen species
MMP mitochondrial membrane potential
DCFH-DA dichlorofluorescein diacetate
DCF dichlorofluorescein
caspases cysteine aspartic proteases
pNA p-nitroaniline
DPI diphenyleneiodonium chloride
Csa cyclosporine A

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Protective Effects of Sesamum indicum Extract Against Oxidative Stress Induced by Vanadium on Isolated Rat Hepatocytes

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ABSTRACT: Vanadium toxicity is a challenging problem to human and animal health with no entirely understanding cytotoxic mechanisms. Previous studies in vanadium toxicity showed involvement of oxidative stress in isolated liver hepatocytes and mitochondria via increasing of ROS formation, release of cytochrome c and ATP depletion after incubation with different concentrations (25–200 μM). Therefore, we aimed to investigate the protective effects of Sesamum indicum seed extract (100–300 μg/mL) against oxidative stress induced by vanadium on isolated rat hepatocytes. Our results showed that oxirigerous extract similar to Alpha-tocopherol (100 μM), different concentrations of extract (100–300 μg/mL) protected the isolated hepatocytes against all oxidative stress/cytotoxicity markers induced by vanadium in including cell lysis, ROS generation, mitochondrial membrane potential decrease and lysosomal membrane damage. Besides, vanadium induced mitochondrial/lysosomal toxic interaction and vanadium reductive activation mediated by glutathione in vanadium toxicity was significantly (P < 0.05) ameliorated by Sesamum indicum extract. These findings suggested a hepato-protective role for extracts against liver injury resulted from vanadium toxicity. © 2015 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2015.

Keywords: vanadium; oxidative stress; sesamum indicum; hepatoprotection; hepatocytes

INTRODUCTION

Vanadium is considered essential for animals; however it has not yet been recognized as a micronutrient for humans (Hosseini et al., 2013). It is used extensively in various heavy industries at toxic levels for industrial workers. Vanadium is involved in the pathogenesis of certain neuro-logical disorders, breathing disorders, cardiovascular diseases, paralyses, and negative effects on the liver and kidneys (Hosseini et al., 2012). It can be found in the environment in algae, plants, invertebrates, fishes, mammals, and crabs and many other species (Aurellano et al., 2009). It was suggested that the health hazards of vanadium are dependent on its oxidation state and pentoxide form of vanadium is more toxic than the elemental form (Zhao et al., 2010). Laboratory data suggested that vanadium can cause harm to the reproductive system of male animals, and also it accumulates in the female’s placenta (Beyermann and Hartwig et al., 2008).
Toxicity of cigarette smoke on isolated lung, heart, and brain mitochondria: induction of oxidative stress and cytochrome c release

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Cigarette smoking is one of the main risk factors for premature human death which is associated with a variety of respiratory and vascular diseases, and cancer due to exposure to hundreds of toxicants. Rat mitochondria were obtained by differential ultracentrifugation and incubated with different concentrations (1%, 10%, or 100%) of standardized cigarette smoke extract (CSE). Our results showed that CSE induced a rise in mitochondrial reactive oxygen species (ROS) formation, lipid peroxidation, and mitochondrial membrane potential (MMP) collapse before mitochondrial swelling ensued in isolated pulmonary mitochondria. Disturbance in oxidative phosphorylation was also confirmed by decrease in ATP concentration in the CSE-treated mitochondria. In addition, collapse of MMP and mitochondrial swelling produced release of cytochrome c via outer membrane rupture or mitochondrial permeability transition (MPT) pore opening. Our results suggested that CSE-induced toxicity in lung tissue is the result of disruptive effect on mitochondrial respiratory chain that leads to ROS formation, lipid peroxidation, MMP decline, and cytochrome c expulsion which results in apoptosis signaling and cell loss.

Keywords: cigarette smoking extracts (CSE); isolated mitochondria; mitochondrial dysfunction; cytochrome c release

Abbreviations

- CSE: cigarette smoking extract
- ROS: reactive oxygen species
- GSH: reduced glutathione
- DCFH-DA: 2',7'-dichlorofluorescin diacetate
- TBARS: thiobarbituric acid reactive substances
- Cs A: cyclosporin A
- MDA: malondialdehyde
- Rh123: rhodamine 123
- BSA: bovine serum albumin
- MPT: mitochondrial permeability transition
- MMP: mitochondrial membrane potential
- BHT: butylated hydroxytoluene
- MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
- AA: antimycin A

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A comparison of toxicity mechanisms of dust storm particles collected in the southwest of Iran on lung and skin using isolated mitochondria

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Dust storms are events of considerable extent and of frequent occurrence in deserts and on their margins. Each year many areas in Iran are affected by dust storms, and the intensity of these storms is higher in the western parts of the country. The toxicity of dust particles partly depends on their concentration. In this study, suspensions containing particles of one, two, and three filters were used and then these particles were dissolved in polar (normal saline) and non-polar (dimethyl sulfoxide) solvents to final concentrations of 2, 5, or 10 mg/ml dust particles for further toxicity assessments. For the assessment of toxicity, rat mitochondria were isolated from lung and skin and then parameters of mitochondrial toxicity including: decreased complex II activity, increased reactive oxygen species (ROS) formation, mitochondrial membrane potential collapse, mitochondrial swelling, and cytochrome c release were evaluated. Our results showed that with the increasing dust concentration (number of filters) mitochondrial ROS formation, mitochondrial swelling, and mitochondrial membrane potential collapse and also cytochrome c release were significantly elevated in both lung and skin tissues compared with related unexposed mitochondria. Our findings suggested that higher particle concentrations lead to more mitochondrial dysfunction, which ultimately ends in either apoptosis or necrosis in both lung and skin tissues. Particles that damage mitochondria probably contain both polar and non-polar such as metal ions components.

Keywords: dust storm; toxicity; mitochondria; lung; skin

Introduction

The concentrations of pollutants released from anthropogenic and natural sources are known to produce adverse effects on human health and the environment according to the World Health Organization report, accounting for approximately 4%—8% of deaths annually in the world related to air pollution (Kathuria 2002; Lopez et al. 2005; Krewski et al. 2005). In recent years, dust storms coming from western neighboring countries drastically increased serious environmental and socioeconomic problems and affecting both western and central parts of Iran. Major dust storms occur over the Middle East region nearly every spring and summer, and produce destructive effects in some countries like Iraq, Saudi Arabia, and Iran (Bocato et al. 2014). The Tigris—Euphrates alluvial plain in the

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