RESEARCH ARTICLE

Luteolin, a Bioflavonoid, Attenuates Azoxymethane-Induced Effects on Mitochondrial Enzymes in Balb/c Mice

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Abstract

Colon cancer (CRC) is a serious health problem throughout the world. Development of novel drugs without side effects for this cancer is crucial. Luteolin (LUT), a bioflavonoid, has many beneficial effects such as antioxidant, anti-inflammatory and anti-proliferative potential. was a potent chemical carcinogen used for the induction of colon cancer. Colon carcinogenesis was initiated by intraperitoneal injection of azoxymethane (AOM) to mice at the dose of 15 mg/body kg weight in Balb/C mice for 3 weeks. Mice were treated with LUT at the dose of 1.2 mg /body kg weight orally. Mitochondrial enzymes such as isocitrate dehydrogenase (ICDH), α-keto dehydrogenase (α-KDH), succinate dehydrogenase (SDH) and the activities of respiratory chain enzymes NADH dehydrogenase and cytochrome c oxidase were found to be elevated in AOM-treated animals. Treatment with LUT decreased the activities of all the parameters significantly. Hence, LUT might be a potent anticancer agent against colorectal cancer.

Keywords: Colon cancer - luteolin - azoxymethane - chemoprevention - mitochondrial enzymes

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Introduction

Colon cancer (CRC) is a serious health problem causes increased mortality every year in worldwide. Highest intake of alcohol, smoking, high fat diets are considered as an etiological factor for the development of CRC. It has three distinct stages including initiation, promotion and progression stages. Multiple signaling alterations were undergoing each stages of the CRC. Therapeutic drugs are designed to target the pathways which is deregulated during cancer will be opt way to deal CRC (Pandurangan, 2013). In general, Natural and synthetic compounds act as crucial chemopreventive agents by modulating oncogenic signals during colon cancer (Sriram et al., 2008; Kumar et al., 2012; Shafie et al., 2013).

Mitochondria are the cellular organelles that provide ATP for metabolism and help to maintain calcium homeostasis in the cell. Damage that compromises key functions may adversely affect continued existence of an organism. Although the mitochondrial electron transport chain is a very efficient system, the very nature of the alternating one-electron oxidation-reduction reactions it catalysis (generating a constantly discontinuous series of 'caged' radicals) predispose each electron carrier to side reactions with molecular oxygen (Hagen et al., 1998). Reactive oxygen species (ROS) may lead to irreversible damage to mitochondrial DNA, proteins, membrane lipids, resulting in mitochondrial dysfunction and finally cell death (Dalton et al., 1999). Some chemical carcinogen primarily attacks mitochondria which is the ''molecular clocks'' in eukaryotes (Pederson, 1978).

Flavonoids are polyphenolic compounds occurring naturally in the plant kingdom, displaying a wide range of pharmacological properties, including anti-carcinogenic and anti-inflammatory (Ross and Kasum, 2002). Luteolin (3', 4', 5, 7-tetrahydroxyflavone) (Figure 1), a flavone subclass of flavonoids is known to be a chemo-preventive effect against malignant tumors *in vivo* without toxic side effects (Yamashita et al., 2000; Ueda et al., 2002). Much attention was focused on luteolin due to its strong antioxidant and radical scavenging property (Shimoi et al., 1994; Ashokkumar and Sudhandiran, 2008). It has anti-proliferative (Ashokkumar and Sudhandiran, 2011) also modulates the status of glycoproteins (Pandurangan et al., 2012), cellular thiols (Pandurangan and Ganapasam, 2013a), membrane bound ATPases (Pandurangan et al., 2013a) and induces apoptosis in AOM-induced colon cancer (Pandurangan and Ganapsam, 2013b). LUT inhibits cell proliferation by controlling wnt/β-catenin signaling in HCT-15 colon adenocarcinoma cells (Pandurangan et

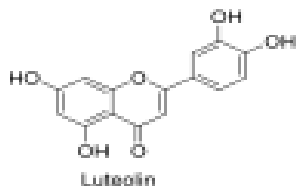

Figure 1. Chemical Structure of Luteolin

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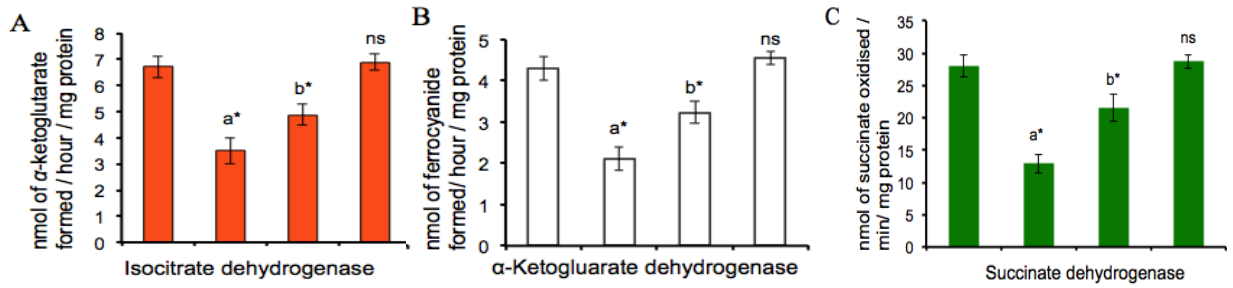

Figure 2. Luteolin Decreased the Activities of ICDH, SDH and α-KGDH. Results are expressed as mean±SD (n=6). p<0.05. Comparisons are made between group 1 (Control) with group 2 (AOM) with group 3 (AOM+LUT). Activities are expressed as nmol of α-ketoglutarate formed/hour/mg protein for ICDH, nmol of succinate oxidised/min/mg protein for SDH, nmol of ferrocyanide formed/hour/mg protein for α-KGDH

al., 2013b). In the present study, the chemopreventive effect of LUT on colon carcinogenesis was investigated in male Balb/c mice by determining the alterations in the mitochondrial enzymes.

Materials and Methods

Chemicals

Azoxymethane was purchased from Sigma Chemicals, (St. Louis, USA). Luteolin was purchased from Cayman Chemicals USA. All other chemicals used in this study were of analytical grade.

Animals

Male Balb/c mice weighing approximately 25-30 g were obtained from the Laboratory Animal Maintenance Unit, Tamilnadu Animal Science and Veterinary University (TANUVAS), Madavaram, India. The animals were acclimatized to the laboratory conditions for a period of 2 weeks. They were maintained at an ambient temperature of 25±2°C and 12/12 h of light-dark cycle and given a standard rat feed (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. The experiments involved with animals were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

Experimental procedure

The animals were divided into four groups (n=6 per group). Group 1 - Control animals received intra peritoneal injections (i.p.) of physiological saline. Group 2 animals were administered with AOM (15 mg/kg body weight) intraperitoneally (i.p.) once in week for three weeks. Group 3 animals (AOM+LUT) were treated with a single dose with 1.2 mg/kg body weight of LUT orally until end of the experiment, after AOM administration as mentioned in group 2 (Ashokkumar and Sudhandiran, 2008). Group 4 received the same dose of LUT alone as mentioned in group 3.

Mitochondria from colon tissue were isolated by the method of Johnson and Lardy, (1967) and the following parameters were analyzed. Protein was estimated by the method of Lowry et al. (1951). Citric acid cycle enzymes isocitrate dehydrogenase (ICDH) was assayed by the method of King (1965), α-keto dehydrogenase (α-KDH) by the method of Reed and Mukherjee, (1969), succinate

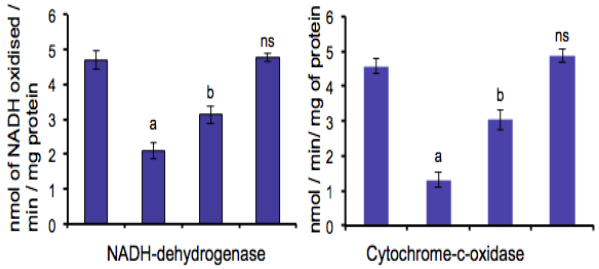

Figure 3. Luteolin Decreased the Activities of NADH Dehydrogenase and Cytochrome-C-oxidase

dehydrogenase (SDH) by the method of Slater and Bonner, (1952). The activities of respiratory chain enzyme NADH dehydrogenase was determined by the method of Minakami et al. (1962) and Cytochrome c oxidase by the method of Pearl et al. (1963).

Statistical analysis

All the data were statistically evaluated with SPSS/10.0 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference (LSD) test *p<0.05 was considered to indicate statistical significance. All the results were expressed as mean±S.D.

Results

Figure 2 shows the activities of the mitochondrial enzymes ICDH, SDH, α-KGDH in the colon of control and experimental groups of animals. The activities of mitochondrial enzymes showed a significant decrease (p<0.05) in group 2 mice. Treatment with LUT (Group 3) altered the activities of these enzymes to near normal levels when compared with AOM-induced animals.

Figure 3 shows the activities of the respiratory marker enzymes such as NADH dehydrogenase and cytochrome-C-oxidase in the colon of control and experimental groups of animals. The activities of respiratory marker enzymes showed a significant decrease (p<0.05) in group 2 mice. Treatment with LUT altered the activities of these enzymes to near normal levels when compared with AOM-induced animals.

Discussion

Colon cancer is considered as a harmful disease

through worldwide. Many compounds from natural sources are nontoxic and very efficient on treating colon cancer (Sriram et al., 2008). Especially plant polyphenols gain a considerable attention in treating many types of cancers including colon cancer. Luteolin in already reported to have a multiple beneficial effects on treating colon cancer. It controls the cancer cell proliferation my modulating the wnt/β-catenin signaling pathway as well as it activates the Nrf2 signaling pathway thereby it offers a protection in colon cancer (Ashokkumar and Sudhandiran, 2011; Pandurangan et al., 2013c). In this study, LUT showed to protect mitochondria by the oxidative-mediated damage during colon cancer. Since plant polyphenols have strong antioxidant activity (Pandurangan et al., 2012b).

The mitochondrial enzymes catalyze the oxidation of several substrates through the TCA cycle, yielding reducing equivalents which are channeled through the respiratory chain for the synthesis of ATP by oxidative phosphorylation. Inhibition of these enzymes by ROS may affect the mitochondrial substrate oxidation, resulting in reduced oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy (Lenaz, 1998). Tumor cell mitochondria can differ structurally and functionally from those of normal cells, but clear evidence in favors of this suggestion is lacking. In the present investigation, AOM-induced mouse showed a significant reduction in the activities of the citric acid cycle enzymes. ICDH refers to the NADP+ dependent enzyme, which in several tissues has dual localization being in part of cytoplasmic and in part of mitochondria. SDH is a marker enzyme in TCA cycle and succinate, phosphate and ATP promote its activity. In this present study, the elevated activities of TCA cycle enzymes during colon cancer were reduced by the subsequent administration of LUT.

NADH dehydrogenase, a flavin linked dehydrogenase passes electrons from NADH to Coenzyme Q. Cytochrome-c-oxidase donates electrons directly to molecular oxygen and constitutes the complex IV. Cytochrome oxidase is the terminal electron acceptor in the chain and must give up its reducing equivalents to permit continued electron transport: if electrons stop flowing through the chain, the proto motive force dissipates and ATP production was cut off. Moreover, it was shown that an increase in mitochondrial ROS production contributes to the decline in the activities of NADH dehydrogenase and succinate dehydrogenase in skeletal muscle of the MnSOD deficient mice (Melov et al., 1999). Decline in cytochrome oxidase activity can cause an increase in H_2O_2 production. It may be speculated that decline in cytochrome c oxidase activity can result in partial blockage of electron flow, which alters reducing potentials of some electron carriers favoring their autoxidation, electron leak and consequent generation of superoxide (Sohal, 1993). This is the direct evidence that mitochondrial respiratory function is impaired under oxidative stress.

Administration of LUT restored the mitochondrial enzymes in group 3 animals. It is well known that, LUT scavenges radical at/near the membrane surface and in the interior of the membranes and these dual effects of LUT could be dependable for its potent LPO inhibitory activity (Samy et al., 2006; Ashokkumar and Sudhandiran, 2008; Pandurangan et al., 2012a). Hence, the role of LUT as a free radical quencher and its role in protecting the mitochondrial membrane stability are apparent from this study. In a nutshell LUT can be act as a potential chemotherapeutic agent against colorectal cancer.

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