Ameliorative effect of green tea against Bisphenol A – induced changes in Succinate dehydrogenase and Adenosine triphosphatase activities: an in vitro study

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Abstract

Bisphenol A (BPA), an environmental estrogen, is widespread in our living environment, because of its production and use increased, exposure of humans to BPA is becoming a significant issue. The aim of the present study was to evaluate the extent to which BPA can affect the liver by measuring hepatic energy metabolism (Succinate dehydrogenase and Adenosine triphosphatase) and its alleviation by hydroalcoholic green tea extract. When liver homogenates were treated with different concentrations (50-250 μg/ml) of BPA, it resulted in significant (p<0.05) and dose-dependent decrease in SDH and ATPase activities. Further liver homogenates were treated with different concentrations (10-50 μg/ml) of green tea extract along with high dose (250 g/ml) of BPA, it resulted in significant (p<0.05) increase in the level of energy metabolism as compared to BPA alone treatment. The effect was dose-dependent. Green tea extracts ameliorated BPA-induced changes, showing maximum protection at 50 μg/ml concentration. Results of the present study indicated that BPA-induced changes in hepatic energy metabolism, which was alleviated by green tea extract due to its phytochemicals having antioxidative properties.

Keywords : Bisphenol A, SDH, ATPase, Antioxidative property and Hepatocytes

Introduction

Exposure of low levels of endocrine disrupting chemicals (EDCs) to human being may be of great concern. They interfere with many metabolic processes and cause widespread damage to body tissue (Humblet et al., 2008). Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane) is one of the endocrine disrupting chemical, used in plastics and food can liners’ manufacture (Willhite et al., 2008). The unbound monomeric BPA can leach out into the surrounding environment (Talsness et al., 2009). The ubiquitous and extensive use of BPA containing products results in a high human exposure worldwide (Vandenberg et al., 2010). It is thought that human exposure mainly occurs through diet as polymers containing BPA can be hydrolyzed under high temperature and acidic or basic drink containers (Welshons et al., 2006). Thus, in humans, BPA is detected not only in serum and urine but also in the placenta and amniotic fluid (Calafat et al., 2005). However recent evidence also indicates that exposure may occur through dermal contact with thermal papers used widely in cash register receipts (Biedermann et al., 2010).

BPA induced numerical chromosomal aberrations and morphological changes in cultured SHE cells (Tsutsai et al., 1998) and in mice it induced achromatic lesions and c - mitotic effects in bone marrow cells
In addition, BPA metabolites were shown to bind to DNA in a cellular system (Edmonds et al., 2004), in cultured SHE cells (Tsutsai et al., 1998), and rodent liver in vivo (Izzotti et al., 2009). Moreover, in estrogen receptor (ER) – positive MCF-7 cells, BPA caused DNA strand breaks that were ER-dependent (Iso et al., 2006). The majority of studies on BPA have focused on their endocrine disrupting and potential adverse effects on the developing reproductive system. Bisphenol A has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes (Nakagawa and Tayama, 2000). In addition, absorption of large amount of BPA through skin has been shown to cause extensive damage to liver, kidney and other vital organs in human (Suarez et al., 2000).

In this study we tested the capacity of green tea extract (GTE) to restore mitochondrial energy deficit induced by BPA. The biological benefits of tea are due to their flavanol content. Tea flavonols are a group of natural polyphenols found in green tea. The main active ingredients of green tea include polyphenolic compounds such as epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG), are popular and are recognized to exert protective effects against bladder hyperactivity (Chen et al., 2009), cancer (D’Alessandro et al., 2003), lipogenesis (Kim et al., 2009), inflammation (D’Alessandro et al., 2003 and Hsu et al., 2007), atherosclerosis (Hsu et al., 2007) and acute liver injury (Lin et al., 2009). EGCG, the primary active component of GTE, exhibited anti-inflammatory, antioxidant and immunosuppressive effects, improved liver dysfunction, reduced liver inflammatory infiltration and hepatocytes apoptosis and abrogated tumor necrosis factor-alpha and interferon – gamma expression at the protein level in plasma (Wang et al., 2006).

EGCG also attenuated hepatic hydroxyproline content and hepatic stellate cell activation, as well as matrix metalloproteinase - 2 activity and protein expression (Zhen et al., 2007). Many in vitro and in vivo studies demonstrated that polyphenols from green tea are anti-carcinogenic by inducing apoptosis and inhibiting cell-growth cyclin-dependent kinase inhibitor and urokinase (Scientific review). Probable mechanisms of action include antioxidant and free radical scavenging activity and stimulation of detoxification systems through selective induction or modification of phase - I and II metabolic enzymes. Present study deals with the evaluation of protective effect of green tea on bisphenol A – induced alteration in energy metabolism levels under in vitro conditions.

Materials and methods

Chemicals and reagents

Bisphenol A was procured from Hi-media Laboratories Pvt. Ltd., Mumbai, India and was of analytical grade. Green tea was purchased from the Brook - Bond Company, Darjeeling green tea, India. All the other chemicals used were of AR grade.

Green tea extract preparation

Green tea extract was prepared according to the method of Bhargava and Singh (1981). Briefly, 5 gm of green tea powder was mixed with 100 ml of 50% aqueous-alcoholic solvent (water and ethyl alcohol mixture). The solution was allowed to stand overnight for maximum extraction of polyphenols. Next-day the solution was filtered through ordinary and then whatman No. 42 filter paper. The filtrate was collected and evaporated below 50°C to obtain final product in the form of residue which was stored under refrigerated condition.

Experimental animals

In the experiment, inbred healthy adult Swiss strain male albino mice weighing 30-35 gm were
obtained from Cadila Research Center, Ahmedabad, India. Animals were kept in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India. They were housed in an air-conditioned room at a temperature of 25°C and 50-55% relative humidity with a 12 h light/dark cycle throughout the experiment. Animals were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and water ad libitum. All the experimental protocols were sanctioned by the Institutional Animal Ethics committee and approved by the Committee for the purpose of Control and Supervision of Experiment on Animals (Reg.– 167/1999/CPCSEA), New Delhi, India. Animals were handled according to the guidelines published by the Indian National Science Academy, New Delhi, India (1991).

Experimental design

Animals were sacrificed and liver was quickly isolated, blotted free of blood, weighed and used for determination of biochemical parameters.

Succinate dehydrogenase (SDH)

SDH activity was assayed by the method of Beatty et al. (1966). The electrons released by the enzyme from the substrate are taken up by 2-(4-iodophenyl) -3(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) which acts as an electron acceptor. The resulted red colored formazan was extracted with ethyl acetate and measured at 420 nm. The enzyme activity was expressed as μgformazon formed/mg t.w.

Adenosine triphosphatase (ATPase) activity

The ATPase activity in the liver was assayed by the method of Quinn and White (1968). ATPase causes hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (i. p). The liberated inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). The optical density was expressed as micromoles of inorganic phosphate released/mg t.w.

Hepatoprotective index

The liver protecting activity of the green tea extract was expressed as hepatoprotective percentage (H) (Prakash et al., 2008) which was calculated using the formula:

\[ H = \frac{T-V}{C-V} \times 100 \]

Where T is the mean value of plant extracts along with the bisphenol A, C is the mean value of bisphenol A alone and V is the mean value of vehicle control animals.

Statistical analysis

The data were statistically analyzed using Graphpad prism software, version 5.03. The results were expressed as the mean ± SEM. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Tukey’s Post-hoc test. The level of significance was accepted with p<0.05. Pearson’s correlation analysis was used to find the correlation between the given concentrations and alteration in hepatic energy metabolism in liver homogenates of mice.

Results

Succinate dehydrogenase (SDH) activity

When liver homogenates were treated with different concentrations (50-250 μg/ml) of BPA, it resulted in significant (p<0.05) decrease in succinate dehydrogenase activity (Fig. 1) as compared to control. The effect was concentration-dependent (17.43%, 28.68%, 43.81%, 57.38%, and 67.41%, respectively, r = 0.99) decrease in succinate dehydrogenase activity with maximum effect at 250 μg/ml concentration.

Co-treatment of BPA (250 μg/ml) along with various concentrations (10-50 μg/ml) of green tea extract to the liver homogenates had significantly
Effect of bisphenol A (BPA) on succinate dehydrogenase (SDH) activity in liver homogenate. Values are expressed as mean ± SEM, n=10. *p<0.05 as compared to control, CON – Untreated control, BPA (50-250 g/ml) – different concentration of bisphenol A.

Ameliorative effects of Green tea extract on SDH activity induced by BPA. Values are expressed as mean ± SEM, n=10. *p<0.05 as compared to control. CON – Untreated control, AC – Antidote control, BPA250 – High dose of (250 g/ml) BPA, GT (10-50 g/ml) – different concentration of GT along with high dose of BPA.

Adenosine triphosphatase (ATPase) activity

ATPase activity was reduced significantly (p<0.05) in liver homogenates by addition of various concentrations (50-250 g/ml) of BPA. The reduction in ATPase activity was 18.39%, 36.46%, 60.71%, 74.38%, and 84.48% respectively (Fig. 3). The effect was concentration – dependent (r = 0.98). Co-treatment of BPA (250 g/ml) along with various concentrations (10-50 g/ml) of green tea extract to the liver homogenates had significantly increased the ATPase activity. Recovery in the activities of ATPase was also achieved by green tea extract treatment which was significant (p < 0.05) and dose-dependent (r = 0.99) as compared to control (Fig. 4). Hepatoprotective index calculated for ATPase activity was 16.76%, 33.71%, 58.12%, 74.24% and 93.06% respectively.
Discussion

In the present study, when liver homogenates were treated with different concentration (50 - 250 μg/ml) of BPA caused significant reduction in activities of SDH and ATPase. The effect was comparatively more pronounced in higher concentration than that of lower concentration.

Succinate dehydrogenase (SDH) is a key enzyme in the mitochondrial Krebs cycle, which is mainly concerned with the aerobic oxidation of acetyl co A and the generation of ATP. Putilina and Eschanko (1969) explained that among the Krebs cycle dehydrogenases, SDH is more active than any other enzyme. Therefore, reduction in aerobic metabolism might be the result of reduced oxygen transport to tissues. A detailed study by Nakagawa and Tayama (2000) explained the relationship between the metabolism and the cytotoxic effects of BPA in freshly isolated rat hepatocytes and isolated hepatic mitochondria. The incubation of hepatocytes with BPA (0.25 – 1.0 mM) elicited a concentration - and time - dependent cell death, accompanied by losses of intracellular ATP and total adenine nucleotide pools. Bisphenol A possesses an inhibitory effect on protein disulfide bonds formation therefore it might have a potential effect in disturbing various physiological functions (Hiroi et al., 2006). The enzymatic hydrolysis of ATP by ATPase is a ubiquitous property of cells which is important for intracellular transfer of energy. Reduction in ATPase activity in liver suggests reduced utilization of ATP produced in the cell. The reduced aerobic oxidation and ATP generation could be responsible for the reduction in ATPase activity.

The result of the present study shows that mitochondria are an important target for the BPA. These compounds cause an uncoupling of the oxidative phosphorylation and inhibit NAD⁺ and FAD⁺ – linked mitochondrial respiration. Treatment of HepG2 cells with BPA for 2 hr leads to a deteriorated mitochondrial architecture. Also in-vitro data have reported that BPA inhibits mitochondrial function (Nakagawa and Tayama, 2000). In this cortex, it is of interest to note that BPA has been reported to interfere with mitochondrial integrity. Nakagawa and Tayama (2000), demonstrated in isolated rat’s liver mitochondria that the intracellular levels of ATP are decreased due to an inhibition of NAD+ and FAD+ - linked respiration. Bindhumol et al. (2003), have also reported a decreased in the activity of antioxidant enzymes which further support impaired mitochondrial functions. Furthermore, it has been shown that BPA induced lipid peroxidation by reactive oxygen species production and oxidative stress which compromised mitochondrial functions (Ooe et al., 2005). When mitochondria are damaged, energy generation in them is inevitably inhibited which contributes to the overall loss in the energy production (Guo et al., 2005).

Green tea extract contains active component EGCG, which has been extensively studied for its anticarcinogenic (Kanwar et al., 2012) and anti-inflammatory (Wu et al., 2012) effects, is a mitochondrial – targeted molecule displaying a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in a variety of neuronal cell types (Schroeder et al., 2009). EGCG has been found to prevent mitochondrial deterioration in aged rat brain (Srividhya et al., 2009), reduce cerebral amyloidosis (Rezai-Zadeh et al., 2005) and correct amyloid – induced mitochondrial dysfunction in a transgenic mice model of Alzheimer disease (Dragicevic et al., 2011). Valenti et al. (2013) shows that green tea treatment (EGCG) renews the capacity to produce energy by restoring the impaired activities of complex I, complex II (SDH), ATP synthase and overall rate of mitochondrial ATP synthesis. Valenti et al. (2013) also indicate that EGCG protects mitochondrial biology. ECGC stimulating
CAMP signaling pathways is able to restore oxidative phosphorylation capacity and promote mitochondrial biogenesis.

Also green tea was more effective in restoring the lipid peroxidation and antioxidant enzymes. Na⁺/K⁺ ATPase is the “-SH” group containing enzymes and is lipid dependent. Reduction in the activity of Na⁺/K⁺ ATPase might be due to enhanced lipid peroxidation by free radicals. Here treatment with green tea increased the activities of Na⁺/K⁺ ATPase this could be due to the ability of green tea extract to protect the –SH group from oxidative damage through the inhibition of peroxidation of membrane lipids.

So it can be concluded, when liver homogenates treated with bisphenol A caused alteration in SDH and ATPase enzymes which also known as energy metabolism, which could be a responsible for hepatotoxicity. Green tea extract reduced bisphenol A induced changes in energy metabolism mainly due to its phytochemicals having antioxidative properties.

References


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