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Vitamin C And E Normalises The Expression Of Sterol Regulatory Element Binding Protein 1c And The Peroxisome Proliferator Receptor In Adipose Tissue Of Pcb-Induced Adult Male Rats

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ABSTRACT

Background:PCB's(PolyChlorinated Biphenyls) are hydrogenated aromatic chemicals that are used extensively in making food packages,water bottles and in plastics of toys thus entering the food chain. Vitamin E and C are the two dietary antioxidants which relieve oxidative stress.

Aim: The role of vitamin C and E on the expression of SREBP 1C and PPARγ mRNA in PCB-induced in experimental animals.

Materials and Method: Male albino wistar rats weighing 180-200 grams were used. Animals were divided into three groups i.e, control group, PCB induced group, PCB induced VitaminC and E treated group consisting of 6 animals each. At the end of treatment the total RNA isolation was done followed by quantification of RNA, cDNA- reverse transcriptase, Quantitative analysis-real time PCR to analyze the mRNA expression levels of SREBP 1C and PPARγ. Data were analysed using one way-ANOVA and significance was considered at the levels of $p<0.05$.

Result:In PCB induced animals there was significant increase in mRNA levels of SREBP 1C conversely PPARγ mRNA expression was found to be significantly increased in PCB exposed rats ($p<0.05$). PCB-exposed rats treated with antioxidant vitamins normalized the altered levels of mRNA expression to that of the control levels suggesting that antioxidant vitamins (Vit C & E) have a protective role against PCB-induced detrimental changes in adipose tissue.

Discussion: Our present findings show that PCB exposure causes detrimental changes in the adipose tissue which may develop diabetes by modulating the expression ofSREBP-1C and PPARγ . Supplementation of Vitamin C and E have protective roles against PCB-induced changes. Hence, Vitamin C and E can be considered for the treatment of diabetes.

Conclusion: Vitamin C and E increases the expression of PPARγ and decreases the expression of SREBP-1C in treated groups and thus reduces lipotoxicity that can reverse insulin resistance. Hence Vitamin C and E can be used as the supplement for diabetes. Studies are needed to further confirm its dosage and mechanism linked to alter the effects of PCB....

Keywords: PCB, vitamin C, vitamin E, oxidative stress, diabetes, SREBP1C and PPARγ, Innovative technology, Novel method.

INTRODUCTION

Polychlorinated biphenyls PCBs are environmental pollutants which have been identified in many different environmental mattresses. Nearly 50% of the PCB mixtures produced across the world are used as capacitors or insulating oils for transformers; others are used in many compound preparations and in manufacturing of toys. High residue levels are detected still in human tissues (Zitko, 1979). PCBs run through the ecosystem in air, water and soil; they also bio-accumulate in the food chain because of their popularity and belong to a glass of chemicals called bioaccumulative toxicants (Falandysz, Tanabe and Tatsukawa, 1994) . This process of increasing toxicity level in higher food chains or Trophic level is called biomagnification. PCB by not only causing bio accumulation it also causes biomagnification. PCBs cause neuronal damage and resulting neuron impairment of motor activities and learning abilities (Sanchezalonso, 2003). Other problems related to PCBs include hypothyroxinemia, memory defects, special learning neurochemical and neuro behavioural alterations and it has adverse effects on the reproductive system too (Latowsky, 1998). PCBs are known to increase reactive oxygen species

(ROS) in neurology cell culture and cause increased neuronal Apoptosis (Liu et al., 2016). PCBs are known to increase oxidative stress. There are two dietary antioxidants that are required by humans called vitamin C and E. vitamin C is also known as ascorbic acid and vitamin E is also known as alpha tocopherol (Ribeiro et al., 2021). Vitamin C is an essential vitamin in various biological functions. Some authors have discussed the effects of vitamin C on cancer treatment (Meyskens, 1995) , sepsis (Zayed et al., 2021) and neuron degenerative disease (Carr and Lykkesfeldt, 2018)(Poranen et al., 1998)(de Smet and Claes, 2013) . Vitamin C also plays an important role in lipid peroxidation as an antioxidant .(Wu et al., 2019),(Chen et al., 2019).Vitamin E is the required nutrients for humans as it helps in prevention of vitamin E deficiency symptoms such as haemolytic anaemia and peripheral neuropathy . Vitamin E is a fat-soluble antioxidant (Li et al., 2020),(Babu and Jayaraman, 2020)).In response to oxidative stress; oxidative stress showed an increased rate of plasma vitamin E disappearance in humans .Our team has extensive knowledge and research experience that has translate into high quality publications,(Malaikolundhan et al., 2020),(Han et al., 2019), (Gothai et al., 2018),(Veeraraghavan, Hussain, et al., 2021),(Sathya et al., 2020),(Yang et al., 2020), (Rajendran et al., 2020),(Barma et al., 2021),(Samuel, 2021),(Samuel et al., 2021),(Tang et al., 2021),(Yin et al., 2021),(Veeraraghavan, Periadurai, et al., 2021),(Mickymaray et al., 2021),(Teja and Ramesh, 2020),(Kadanakuppe and Hiremath, 2016) (Vijayakumar et al., 2010; Kavitha et al., 2014; Lekha et al., 2014; Sahu, Kannan and Vijayaraghavan, 2014; Neelakantan et al., 2015)

The aim of the study is to analyse the role of vitamin C and E in altering the oxidative stress induced by PCB in experimental animals.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. PCB was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. SREBP 1C, PPARγand β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

Animals

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/12.2019/048). Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha Dental College and Hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. Rats were divided into 3 groups, each consisting of 6 animals.

Experimental Design

Group 1: Control (Vehicle control, rats were intraperitoneally (i.p.) administered

with the vehicle (corn oil) for 30 days.

Group 2: Rats received PCB (PCB was dissolved in corn oil at a dose of 2mg/kg

body weight (b.wt) intraperitoneally daily at 10:00 a.m. for 30 days.

Group 3: PCB and vitamin E (dissolved in olive oil at a dose of 50 mg/kg body

weight), and vitamin C treated (100 mg/kg body weight dissolved in distilled water daily at 10 AM through gastric intubation for 30 days).

At the end of treatment, animals were anesthetized with sodium thiopental (5 mg/kg, i.p), and 20 ml of normal saline was perfused through the left ventricle, to clear blood from the liver, and other organs. Visceral adipose tissue was dissected out and used for the assay of various parameters.

Gene expression analysis by Real Time-PCR

Isolation of total RNA

Total RNA was isolated from control and experimental samples using TRIR (total RNA isolation reagent) kit. Briefly, 100 mg fresh tissue was homogenized with 1 ml TRIR and the homogenate was transferred immediately to a microfuge tube and kept at -80°C for 60 min to permit the complete dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was added, vortexed for 1 min and placed on ice at 4°C for 5 min. The homogenates were centrifuged at 12,000 x g for 15 min at 4°C. The aqueous phase was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 sec and placed on ice at 4°C for 10 min. The samples were centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was discarded and RNA pellet was washed with 1 ml of 75% ethanol by vortexing and subsequent centrifugation for 5min at 7,500 x g (4°C). The supernatant was removed and RNA pellets were mixed with 50 µl of autoclaved Milli-Q water and dissolved by heating in a water bath for 10 min at 60°C.

Quantification of RNA

Diluted RNA samples were quantified spectrophotometrically by measuring the absorbance (A) at 260/280 nm. 40 µg of RNA in 1 ml gives one absorbance at 260 nm. Therefore, the concentration of RNA in the given sample can be determined by multiplying its A260 by 40 and dilution factor. The purity of RNA preparation can be calculated using the ratio between its absorbance at 260 and 280 nm. A ratio of absorbance at 260/280 nm> 1.8 is generally considered as good quality RNA (Fourney et al., 1988). The purity of RNA obtained was 1.8.

Reverse Transcriptase – Polymerase Chain Reaction (RT – PCR)

RT-PCR is an approach for converting and amplifying a single stranded RNA template to yield abundant double stranded DNA products. 1. First strand reaction: Complementary DNA (cDNA) is made from the mRNA template using Oligo dT, dNTPs & reverse transcriptase. 2. Second strand reaction: After the reverse transcriptase reaction is complete, standard PCR (called the "second strand reaction") is initiated. Principle RT-PCR is a method used to amplify cDNA copies of RNA. It is the enzymatic conversion of mRNA into a single cDNA template. A specific oligodeoxynucleotide primer hybridizes to the mRNA and is then extended by an RNAdependent DNA polymerase to create a cDNA copy. First strand DNA synthesis The RT kit was purchased from Eurogentec (Seraing, Belgium). Reagents 1. 10X RT buffer: One vial containing 1.4 ml of 10X RT buffer. 2. EuroScript reverse transcriptase: One tube containing 75 µl of Moloney Murine leukemia virus reverse transcriptase (3750 U at 50 U/µl).

Quantitative Real Time PCR:

The purpose of a PCR (Polymerase Chain Reaction) is to make a huge number of copies of a gene. There are three major steps in a PCR, which are as follows: Denaturation at 94°C for 3 min: During the denaturation at 94°C for 2-5 min, the double strand melts open to single stranded DNA, all enzymatic reactions stop. Annealing at 54°C- 65°C for 30 sec: Ionic bonds are constantly formed and broken between primer and the single stranded template to ensure the extension process. Extension at 72°C for 30 sec: Primers that are in positions with no exact match get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP from 5' to 3', reading the template from 3' to 5' side; bases are added complementary to the template). Because both strands are copied during PCR, there is an exponential increase of the number of copies of the gene. Reagents used for PCR amplification are : 1. 2X Reaction buffer: The PCR master mix kit was purchased from Takara Bio Inc., Japan. Contains TaKaRa Ex Taq HS (a hot start PCR enzyme) dNTP Mixture, Mg^{2+}, Tli RNase H (a heat-resistant RNase H that minimizes PCR inhibition by residual mRNA), and SYBR Green I. 2. Forward primer (10µM) 3. Reverse primer (10µM) 4. cDNA- Template 5. Autoclaved milli Q water 6. Primers: The following gene specific oligonucleotide primers were used. Details of primers used in the present study are: Rat PPARγ- FW: 5'-GGACGCTGAAGAAGA-3'; RW: 5'-GACCTG CCGGGTCCTGTCT GAGTATG-3'- Rat SREBP-1c- FW: 5'- GGAGCCATGGATTGCACATT -3'; RW: 5' – AGGAAGGCTTCCAGAGAGGA -3'; Rat β-actin; FW – 5'- TACAGCTTCACCACCACAGC - 3'; RW– 5'-TCTCCAGGGAGGAAGAGGAT - 3'.

Procedure

Procedure Real Time PCR was carried out on CFX 96 Real Time system (Bio-Rad). The reaction mix (10 µl) was prepared by adding 5 µl of 2X reaction buffer, 0.1 µl of sense and antisense primer, 1 µl of cDNA and 3.8 µl of sterile water. The thermal cycler protocol was as follows: Initial denaturation at 95˚C for 3 min, followed by 40 cycles of PCR, denaturation at 95˚C for 10 sec, annealing at 60˚C for 20 sec and extension at 72˚C for 20 sec. All reactions were performed in triplicate along with no template control (NTC). Melt curve analysis was performed using the thermal cycling programmed at 50-95˚C for each sample to determine the presence of multiple amplicons, non-specific products and contaminants. The results were analysed using CFX 96 Real Time system software (Bio-Rad). As an invariant control, the present study used rat β-actin.

Statistical analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± SEM. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism version 5. The results with $p<0.05$ level were considered to be statistically significant.

RESULTS

Effect of antioxidant vitamins (Vitamin & E) on SREBP1-c mNRA expression

In the controlled group level of SREBP 1C mRNA is noted. For PCB Induced groups the SREBP 1C mRNA value is as high as 1.5 and in PCB induced and vitamin E and C treated groups the SREBP 1C mRNA levels reduces as that of the control group (fig 1).

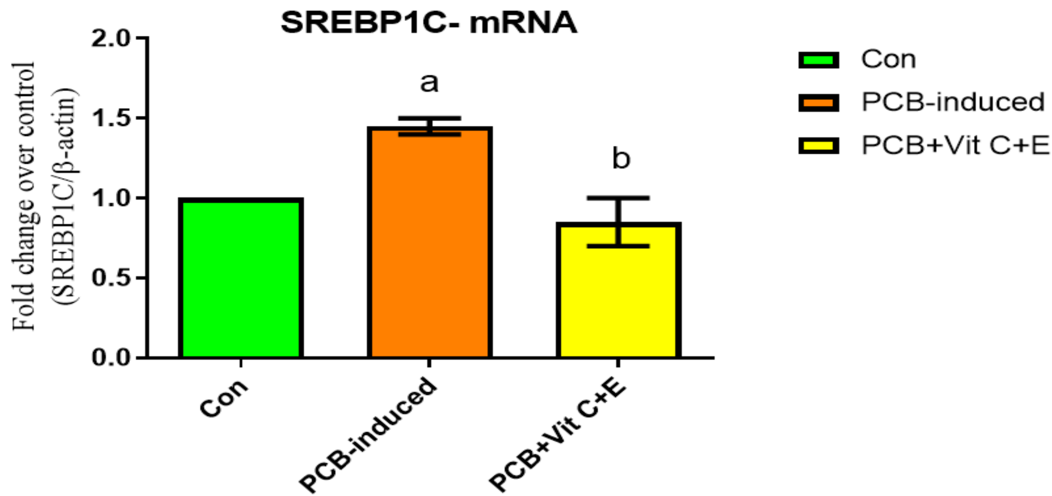

Fig.1: Effect of antioxidant vitamins (Vit E and C) on the expression of SREBP-1C in adipose tissue of PCB-induced rats. SREBP-1c mRNA expression was assessed by Real Time-PCR. Each bar represents Mean S.E.M of 3 observations representing 6 animals. Significance at $p<0.05$. a-compared with control; b-compared with PCB-induced.Green bar represents expression level of control rats, orange bar represents expression level of PCB-induced rats, and yellow bar represents expression levels of PCB-induced rats which were later treated with Vitamin C and E.

Effect of antioxidant vitamins (Vitamin & E) on PPARγ mNRA expression

In the controlled group level of PPAR γ ids are noted. For PCB induced groups the PPAR γ mRNA value reduced significantly and in PCB induced and vitamin C and E treated groups the PPAR γ levels increased to the normal levels (fig 2).

FIGURE 2:

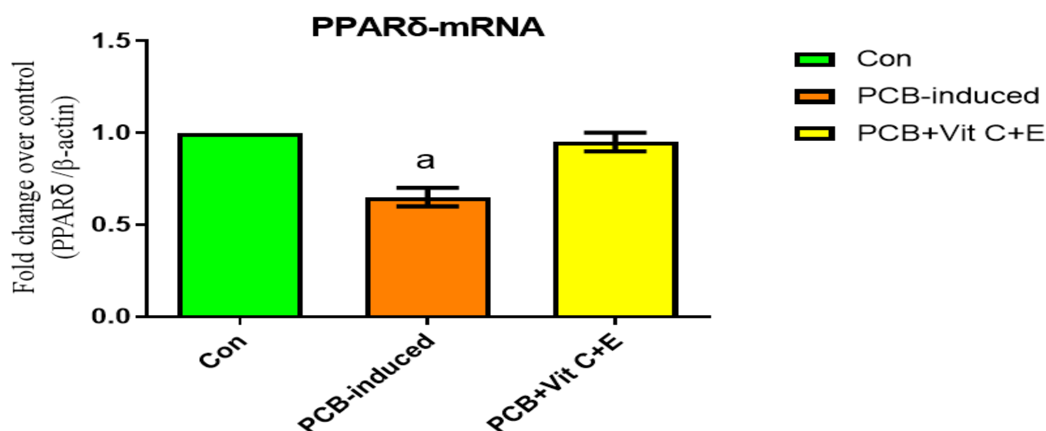

Fig2: Effect of antioxidant vitamins (Vit E and C) on the expression of PPAR γ in adipose tissue of PCB-induced rats. PPAR γ mRNA expression was assessed by Real Time-PCR. Each bar represents Mean S.E.M of 3 observations representing 6 animals. Significance at $p<0.05$. a-compared with control; b-compared with PCB-induced.Green bar represents expression level of control rats, orange bar represents expression level of PCB-induced rats, and yellow bar represents expression levels of PCB-induced rats which were later treated with Vitamin C and E.

DISCUSSION

Polychlorinated biphenyls (PCBs) are tenacious natural toxins with long half-lives in the human body, which are said to increase oxidative stress and other health problems such as diabetes and they may go about as endocrine disruptors and show

endocrine framework impacts (Müllerová and Kopecký, 2007). Longnecker et al (Longnecker *et al.*, 2001) uncovered a 30% more elevated level of complete PCBs in diabetic (fundamentally type 1) pregnant ladies than in nondiabetic subjects enlisted in 1959–1966 (Satyanarayana *et al.*, 2015) . Comparative ends were drawn from the National Health and Nutrition Examination Survey (NHANES) and other populace concentrates in Belgium (Fierens *et al.*, 2003) and Seveso, Italy (Vasiliu *et al.*, 2006). This information raised incredible worries for general wellbeing and advanced etiological examination into the natural impacts of POPs (Cranmer *et al.*, 2000). The cross-sectional investigation discoveries warrant a subsequent accomplice study to evaluate the drawn out impacts of POPs on the danger of creating diabetes and hypertension (Jayashree *et al.*, 2013) . A mass harming happened in focal Taiwan after an amount of rice-grain oil ingested in 1978–1979 was subsequently discovered to be debased with PCBs and their warmth corrupted results (Hsu *et al.*, 1985) . PCB causes various effects in our body which can be altered with vitamin C and vitamin E (Williams *et al.*, 2013). The levels of SREBP 1C mRNAin PCB induced group of rats increased(Ponnulakshmi *et al.*, 2019) while in the PCB induced and vitamin E and C treated group of rats the values or the levels of SREBP 1C reduced significantly as that of the control group . The levels of PPAR γ significantly reduced in the PCB-induced group of rats and in vitamin E and C treated groups of rats the value increased as that of the control group. Low PPARγ expression reduces the capacity of adipose tissue to store fat resulting in lipotoxicity (Arunachalam, Tirupathi Pichiah and Achiraman, 2013). Over-expression of the SREBP-1C gene induces cholesterol metabolism (Meng *et al.*, 2019) and results in fatty liver and leads to insulin resistance and thus causes Diabetes mellitus (Selvaraj *et al.*, 2009) .

CONCLUSION:

In this study it is evident that PCB induced groups have diabetes which is shown with the help of inflammatory markers. Vitamin C and E increases the expression of PPARγ and decreases the expression of SREBP-1C in treated groups and thus reduces lipotoxicity that can reverse insulin resistance. Hence Vitamin C and E can be used as the supplement for diabetes. In future, more studies are needed to further confirm its dosage and mechanism linked to alter the effects of PCB.

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CONFLICT OF INTEREST:

The authors hereby declare that there is no conflict of interest in this study.

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AUTHORS CONTRIBUTION:

A (Ashwin Krishna B) - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B (Gayathri.R) - contributed in study design, guiding the research work, manuscript correction.

C, D, E (J. Selvaraj V, Vishnu priya, Kavitha.S) - study design, statistical analysis, manuscript proofreading and correction...

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