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ORIGINAL PAPER

Tocopherol Moderately Induces the Expressions of Some Human Sulfotransferases, which are Activated by Oxidative Stress

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Abstract

Oxidative stress is generated in biological system by several endogenous/exogenous factors like environmental-pollution/toxicity/diseases and by daily-life-stress. We previously showed that oxidative-stress impaired the activities/expressions of phase-II drug-metabolizing enzyme, sulfotransferases (SULTs). The SULT catalyzes sulfation of endogenous/exogenous compounds. Vitamin E is globally consumed by a large number of individuals for the cellular protection from oxidative stress and aging. Here, vitamin E (tocopherol; α/γ and tocotrienol; α/γ; 0, 1, 10, or 100 μM) was tested in human carcinoma cell line, HepG2 for their influences on SULTs expression/(western blotting). The effects of oxidant (glutathione-oxidized/GSSG) or reductant (glutathione-reduced/GSH, Dithiothreitol/DTT) on SULT activities were studied in rat-liver/human intestinal tissues. Results suggest, tocopherol is more inductive to monoamine-SULT (MPST) and Dehydroepiandrosterone-SULT (DHEAST) compared to that of tocotrienol (inconsistent change in PPST, phenol sulfotransferase/MPST/EST, estrogen sulfotransferase). The nuclear-factor constitutive androstane receptor (CAR) was found to be induced moderately. This study overall describes that vitamin E moderately influences SULTs expression. The induction ability of tocopherol should be judged taking into account its long-term consummation. Oxidative stress activates rat and human SULTs activities and expressions. Further studies are necessary in this regard.

Keywords Human sulfotransferases · Tocopherol and tocotrienol · Oxidative stress · SULT induction · Western blot

Introduction

Stress is the driving force for the adaptation and evolution process [1]. In wider sense, the environmental stress alters several physicochemical properties of the biological systems [2]. Oxidative stress is generated due to the redox imbalance in the cell [3]. These imbalances are initiated by the free radicals, lipid peroxides, transition metals, hydrogen peroxides, and in some conditions due to low antioxidant status, higher ratios of oxidized to reduced compounds (i.e., NAD/NADH+, GSSG/GSH) [4]. Regulation of sulfation of several endobiotics (i.e., bioamines, phenols, steroids, and vitamins) and xenobiotics (food nutrients, micronutrients natural, or prescription drugs) have great impact in the physiological and pathological conditions [5]. Sulfation-mediated polarization of biomolecules may result in higher bioavailability, excretion, and biotransformation/bioactivation of the compound [6]. A group of phase-II enzymes; namely sulfotransferases (SULTs) are recruited for sulfation-mediated modification of endobiotics and xenobiotics [7]. Cysteine present in the active/catalytic site of some SULT isoforms makes that enzyme sensitive to the redox-state of the cell [8]. Redox mediated modifications of SULT activity has been demonstrated earlier [9]. In our previous investigation we showed that several stress and oxidative stress could modify active Cys in SULTs. This has physiological and pathological implications [10].

In our previous report, we showed that retinoic acid (vitamin A) and folate had different degrees of induction effects on SULTs protein/gene expression [11–13]. This

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suggests that micronutrients are capable to induce SULTs expressions though those are consumed with a very low amount. Vitamin E and its different derivatives (α, β, γ-tocopherol) are widely consumed in natural forms (fruits and vegetables) and also in synthetic forms [14]. This vitamin is protective in cellular stress generated due to free-radical loading in the cells [15]. As it is explained earlier about the stress activation of SULTs function, it is of great importance to study whether the antioxidant vitamin E has any role in SULTs expression. Possible alterations of SULTs expressions/activities by vitamin E could be influential for the sulfation-mediated drug metabolism. Taken into account the fact of oxidant-induced SULTs regulations, the present objective of the study is important.

In the current study, we investigated the effect of vitamin E on SULTs expressions. Present study suggests that vitamin E especially in the form of tocopherol moderately induce the protein expressions (20–40%) of monoamine catalyzing sulfotransferase (MPST) and dehydroepiandrosterone catalyzing sulfotransferase (DHEAST) in human HepG2 cells. In this study, oxidative stress (oxidized environment, GSSG) has been shown to suppress and reductant (GSH, DTT) to induce the SULTs activities.

Materials and Methods

Experimental Procedure

ρ-nitro-phenyl sulfate (PNPS), β-naphthol, [14C] β-naphthol (4.7 mCi/mmol), [1,2,6,7-3H(N)] dehydroepiandrosterone ([3H]DHEA, 60 Ci/mmol), and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) are purchased from Sigma-Aldrich (St. Louis, MO). SDS-PAGE reagents are procured from BioRad (Hercules, CA). Nitrocellulose membrane (Immobilon-P; Millipore Corporation, Bedford, MA) is purchased from Fisher Scientific (Fair Lawn, NJ). Western blot chemiluminescence reagent kits are procured from Pierce Chemical (Rockford, IL). Rabbit anti-PPST, DHEAST, MPST, and EST (phenol catalyzing sulfotransferase, monoamine catalyzing sulfotransferase, dehydroepiandrosterone catalyzing sulfotransferase, and estrogen catalyzing sulfotransferase, respectively) antibodies are supplied by Panvera (Madison, WI) Protein determination kit is procured from BioRad. All other reagents are of highest and analytical grade.

Tissue Cytosols and Drug Treatment

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) ~11 weeks old (~200 body weight) are used in this study. Rats are acclimatized for at least 1 week at its standard housing temperature and humidity. Those are supplied with rodent chow and water. Fresh cytosols are prepared from rat liver. In case of animal experiment, all applicable international, national, and institutional guidelines are followed regarding the care and use of animals. Human liver and intestinal samples are collected from the hospital resources (operated patients) and those are used for cytosol preparation. The cytosol is incubated with oxidant (GSSG) or reductant (GSH and DTT) and after the incubation period those are tested for SULTs activities.

All procedures related to human samples are in accordance with the ethical standards of the institutional and/or national research committee. At every step the regulations are followed as proposed in 1964 Helsinki declaration and its later amendments. All experimental protocols are approved by the institutional ethical committee.

Cell Culture and Drug Treatment

Human hepatocarcinoma HepG2 cells are collected from the American Type Culture Collection (ATCC, Manassas, VA). The cell is maintained in Dulbecco's Modified Eagles's Medium Nutrient Mixture F-12 Ham (Sigma). The media is supplemented with L-glutamine and 15 mM HEPES, and 10% foetal bovine serum and incubated in standard conditions (37 °C in a humidified incubator containing 5% CO_2, 95% air). Cells are seeded in 10 cm plates at a density of 5×10^6 cells per plate [16–18]. After seeding on day 1, vitamin E is added (tocopherol; α/γ and tocotrienol; α/γ all with 0, 1, 10, or 100 μM) to the medium in separately marked plates. Control plates are added to the vehicle. The medium is refreshed every 3 days with the new addition of drug. On day 10 the cells are harvested and the cytosols are prepared.

Cytosolic Sample Preparation

Cytosol from liver homogenates are prepared with 50 mM Tris buffer containing 0.25M sucrose, pH 7.5. Mucosal homogenates from intestinal tissues are prepared with the same buffer containing 0.01 mg/ml trypsin inhibitor and 10 μg/ml phenyl methylsulfonyl fluoride (PMSF) [11]. For HepG2 cells, the cell pellet is dissolved and homogenized in 1000 μL lysis buffer (150 mM NaCl, 20 mM Tris, 1 mM EDTA, 1 mM DTT, 0.1 mg/ml trypsin inhibitor, 0.3% Tween-20 (V/V), and 1 mM PMSF). The homogenate is centrifuged at $100{,}000 \times g$ for 1 h at 4 °C. The cytosol aliquots are preserved at 80 °C for enzymatic assay and western blot.

Enzyme Assays

Two different enzyme assay methods are used. All enzymatic assays are run from five independent experiments and the data are utilized to calculate the mean and corresponding SD values.

PNPS Assay

In the current study liver cytosols is used to determine the β-Naphthol sulfation activity [19]. Briefly, sulfation activity is screened in a reaction mixture containing 50 mM Tris buffer [pH 6.2] containing 5 mM PNPS, 20 µM PAPS, and 0.1 mM β-naphthol. The 50 µg proteins from the rat-liver cytosols are used as the enzyme source. After 30 min of incubation at 37 °C in a shaking water bath the reaction is stopped by adding 250 µl of 0.25M Tris. The reaction mixtures are read at 401 nm in a spectrophotometer [9].

Radioactive Assay

The reaction conditions and their ingredients are same as described above in the PNPS assay. The *β*-Naphthol sulfation activity in intestinal cytosols and DHEA-sulfation activities in HepG2 cell are assayed by the radioactive assay method previously described [20, 21]. For the determination of intestinal β-naphthol sulfation activity, (14C) *β*-naphthol (4.7 mCi/mmol; 0.1 mM final concentration) is used as the substrate. To assay the DHEA-sulfation activity, (3H) DHEA (finally 0.4 Cµ/mmol; 2 µM) is used as the substrate. In all types of assay protocols, 20 µM PAPS is used. Liver cytosolic protein (50 µg) is used as enzyme source in a final reaction volume of 250 µl. The reaction is stopped by adding 250 µl (0.25 M, pH 8.7) of Tris buffer after 30 min of incubation (37 °C) in a shaking water bath. Then these reaction mixtures are extracted twice by successive addition of 0.5 ml of water-saturated chloroform [9]. After the final extraction, 100 µl of aqueous phase is collected for scintillation counting. The data shown in the figures are the average of five independent sets of data collected from five different animals. PAPS is eliminated from the controls of both assay methods.

Estrogen Sulfotransferase Enzyme Activity Assay

The HepG2 cell cytosol (50 µg protein from each group) is employed to determine hSULT1E1 (EST) activity by the radioactive assay method [21–24]. [3H]E2 (0.9 Ci/mmol; 0.15 µM to make the final concentration) is used as substrate in the reaction mixture. For all of the assays, 20 µM PAPS in 250 µl reaction mixture are used containing 50 mM Tris buffer (pH 6.2). The reaction continued for 30 min incubation in a shaking water bath (37 °C) and then it is stopped by adding 250 µl of 0.25 M Tris (pH 8.7). The extraction process is performed twice by adding a total 1 ml of water-saturated chloroform. After the final extraction, aqueous phase is used for scintillation counting. The data collected from the enzymatic assay from each protein source.

SULT Inactivation by GSSG and Activation by GSH and DTT

Samples containing human intestine cytosol (final concentration, 1.0 mg/ml) and rat-liver cytosol are assayed for the evaluation of *β*-naphthol sulfation activity. The enzyme sources are incubated in Tris buffer (pH 6.2) at RT with different concentrations of GSSG or GSH or DTT as demonstrated in the figures. Aliquots (50 µl) of the mixture are used to determine respective substrate sulfation activity as described in earlier paragraph. The proper control test is conducted by adding an equal volume of water in the reaction mixture.

Western Blot Analysis for SULTs Expression Study

Three independent experiments are conducted for all western blot analysis and the representative images are provided. The densitometry analysis results are represented as the mean and SD values of the corresponding blot.

Liver cytosol protein (10 µg) and HepG2 cell cytosol protein (25 µg) are used in 10% PAGE system (BioRad). After running the protein bands are transferred onto a nitrocellulose membrane. For rat liver and HepG2 cells, membranes are incubated with rabbit anti-hPPST or hMPST or hDHEAST or hEST (1:5000 to 1:2000 concentration) in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, and 0.05% (v/v) Tween-20) containing 5% (w/v) dried milk for 2 h. Then, membranes are washed several times and incubated in secondary antibody (horseradish peroxidase-conjugated Immuno-Pure goat anti-rabbit IgG; H + L) at 1:5000 dilutions in the same buffer for 2 h. The membranes are washed in TBST and finally in phosphate-buffered saline. Fluorescent bands are developed with 1 ml of substrate containing the same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at RT. The X-ray films are exposed to the membrane and then developed. Films are scanned and the densitometry analysis is performed in a Gel Documentation and Analysis System from Advanced American Biotechnology and with AAB software (Fullerton, CA) [22].

Bioinformatics Study

Sequence collection multiple sequence alignment

The protein sequence of several SULTs are collected from PubMed https://www.uniprot.org and (https://www.ncbi.nlm.nih.gov/pubmed/). The alignment of collected sequences is done in Multalin Interface (http://multalin.toulouse.inra.fr/multalin/). Different types of alignment studies are

performed utilizing available SULTs sequences and the homology assessment is done.

PDB structure collection (3D structure)

The 3D structure is obtained from Protein Data Bank (https://www.rcsb.org/), and those are visualized by PyMol (PyMOL Molecular Graphics System) or RasMol (RasMol 2.7.5, Molecular Graphics Visualization Tool) Swiss model (https://swissmodel.expasy.org/) which we have generated in Swiss model.

Generation of unavailable 3D structure in PDB format and superimposition on bound factors

The unavailable 3D structures of some proteins are designed (~2.00 A°) by Swiss model (https://swissmodel.expasy.org/interactive) utilizing their UniProt sequence and using available human template PDB structures. The PDB format for the newly developed structure are then downloaded and viewed in Rasmol for superimposition purpose. Newly generated structures are screened to evaluate protein structure, α-helix, β-sheets, and amino acid locations of active site, metal binding site, cysteine, and possible disulfide bridges. To find the occurrence rate and the conserved nature of Cys residues, alignment (Multalin software (http://multalin.toulouse.inra.fr/multalin/) is performed. The software Molscript (http://www.csb.yale.edu/userguides/graphics/molscript/use.html) and Raster-3D (https://bio.tools/Raster3D) are also utilized to stabilize and superimposition of the picture.

The pdb structures of SULTs are critically analyzed to evaluate their substrate/ligand binding locations/Cys positions for possible redox regulation provisions, and how much they are close to that ligand binding sites. The biochemical experiments are correlated to the bioinformatics data to predict the possible redox regulations of the Cys residues on the enzymatic catalytic activities.

Statistical Analysis

Student's t test is performed to calculate the statistical significance with the difference between means control and vitamin E-treated rats. Data presented in the figures denote mean ± SD of the results collected separately from three individual animals. Data are the representation of six independent experiments.

Results

The present investigation demonstrates that oxidative stress can change the intracellular redox environment. The Fig. 1 explains that human and rat tissue cytosols and purified SULT activities are decreased by oxidants (GSSG) and increased by the reducing agents (GSH, $p < 01$, or DTT, $p <$ 001). And this alteration was found to be 70–120% of the control. The presence of active Cys residues in some of the SULTs like 1A1 or ASTIV, 2A1 or STa, and 1E1 are the main reason for SULTs redox modifications (Fig. 2). This is shown in the Fig. 2 [11, 13, 18, 20]. These Cys are very close to the ligand binding sites of the corresponding

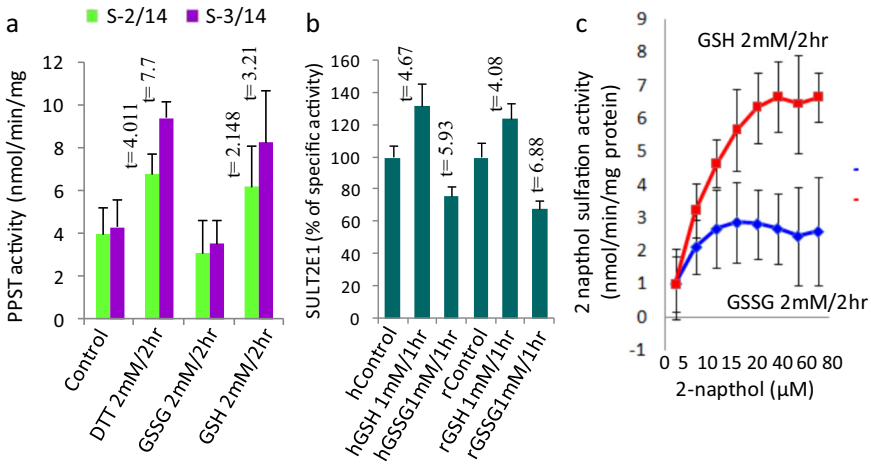

Fig. 1 Phenol catalyzing PPST (SULT1A1) specific activities in human intestinal tissues are shown to be affected in their catalytic efficiency by oxidant (GSSG) and promoted by reductants (GSH and DTT) (**a**). Rat and human E2 sulfation capabilities as percentage of activities (compared to control) in pre-incubated SULT2E1 with GSSG or GSH are shown in figure (**b**). *Michaelis–Menten* kinetics of 2-napthol sulfation by rat ASTIV is influenced by GSSG and GSH; when GSH and DTT increase the enzyme activity, GSSG decrease the activity (**c**). Graph and diagram presented in figures are the mean ± SD of five independent experiments. Level of significance are calculated after comparing with corresponding control at df = 8. p values are; $p = 0.05$ when $t = 2.306$, $p = 0.01$ when $t = 3.355$, $p = 0.001$ when $t = 5.04$

enzyme and upon modifications by the redox environment this Cys interferes with the enzymatic activities (Fig. 2). Rat phenol sulfating enzyme PPST/ASTIV (aryl sulfotransferase, rat isoform, and equivalent to phenol sulfotransferase) was incubated with GSH or GSSG and activity was assessed in different 2-napthol concentration and found that activity consistently decreased (~3-fold) in GSSG incubation (Fig. 1c). This event generated an earlier plateau with comparison to a typical Michaelis–Menten graph. It may have importance and consequences in physiological and pathological conditions.

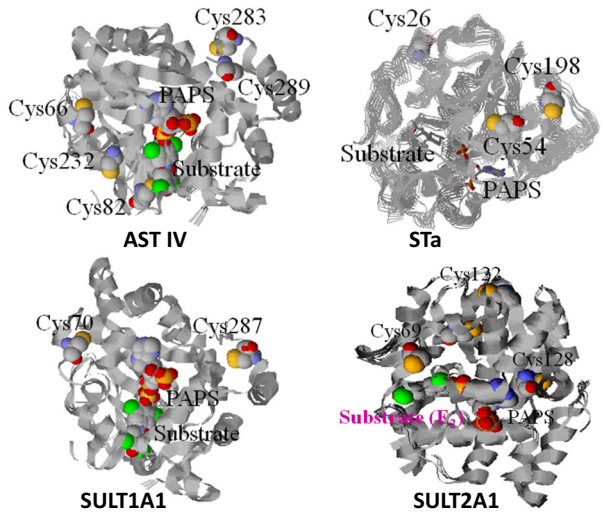

Fig. 2 Four different isoforms of rat and human sulfotransferases are presented as ribbon from PDB source. Enzymes are shown to be bound with PAPS and the corresponding substrate. The Cys residues in the protein sequences are also presented to be close enough in phenol catalyzing ASTIV, SULT1E1, and in estrogen catalyzing SULT2E1

In our earlier studies, we showed that several drugs and micronutrient interferes SULTs activity [19, 20]. In this study, we demonstrated that some of the isoforms of vitamin E can moderately induce the expression of SULTs in human HepG2 cells. Alfa and gamma tocopherol were found to induce more SULTs especially MPST and DHEAST. But the gamma tocopherol was found to be more responsive to SULTs expression (Fig. 3). The 10 and 100 μM of gamma tocopherol increased DHEAST expressions. The expression pattern between MPST and DHEAST was little different. When MPST and DHEAST are compared, it can be said that these two enzymes proteins have different structures and amino acids sequences. And the tocopherol interacted to them are of two categories α and γ. So, the possible interactions of two isoforms (vitamin E) with two different SULTs assumably will not be similar. Moreover, if the vitamins are effective at transcriptional and or translational levels then also the results could be different. Regarding the decrease of MPST at higher dose of γ tocopherol, it may be interpreted that any physical interaction does not occur maintaining linearity at the extremely higher concentration of the drugs, it reaches to a plateau and even some time it may demonstrate a certain degree of inhibition. These two enzymes' protein sequences have different number of Cys residues at different locations. Both the alfa and gamma forms of tocopherol did not influence the PPST and EST expressions (Fig. 3).

In Fig. 4, we demonstrated the SULTs responses against the exposure of tocotrienol of both isoforms. In this case, PPST was found to be induced by α form of Vit E but MPST was increased by the γ form at the highest dose only (100 μM). The protein expressions were noticed in Fig. 4a and their densitometry analysis data are presented in Fig. 4b. In several drug induction mechanisms the roles of

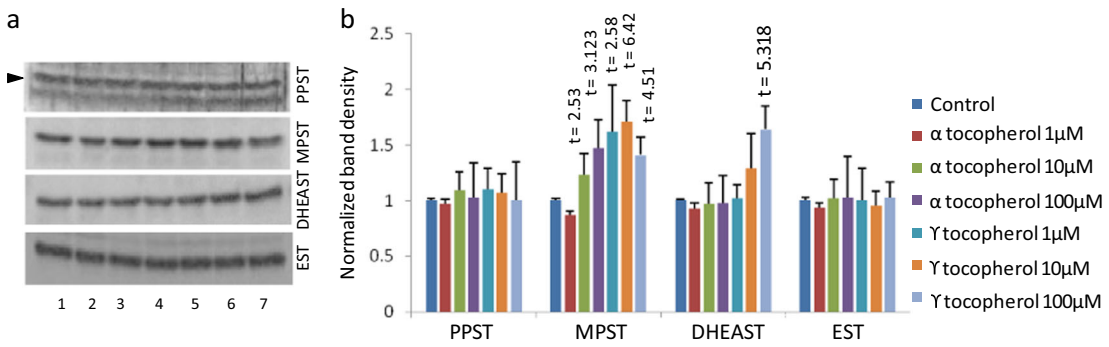

Fig. 3 Representative blot shows human SULTs expression in response to vitamin E (tocopherol) exposure in HepG2 cells (**a**) and those desitometry analyses is plotted as bar plot (**b**) from multiple repeat experiments. PPST phenol catalyzing sulfotransferase, MPST monoamine catalyzing sulfotransferase, DHEAST dehydroepiandrosterone-catalyzing sulfotransferase, EST estrogen catalyzing sulfotransferase. In fig. **a** lane distribution is as follows, 1—control, 2, 3, 4 are 1, 10, and 100 μM of α-tocopherol, respectively, and 5, 6, 7 are γ-tocopherol of similar sequence of dose. Fig. **b** densitometric data and their normalized values are presents in different groups of four SULTs. Data are presented as mean ± SD of three independent experiments. Level of significance are calculated after comparing with corresponding control at df = 4. p values are; $p = 0.05$ when $t = 2.7764$, $p = 0.01$ when $t = 4.604$, $p = 0.001$ when $t = 8.61$

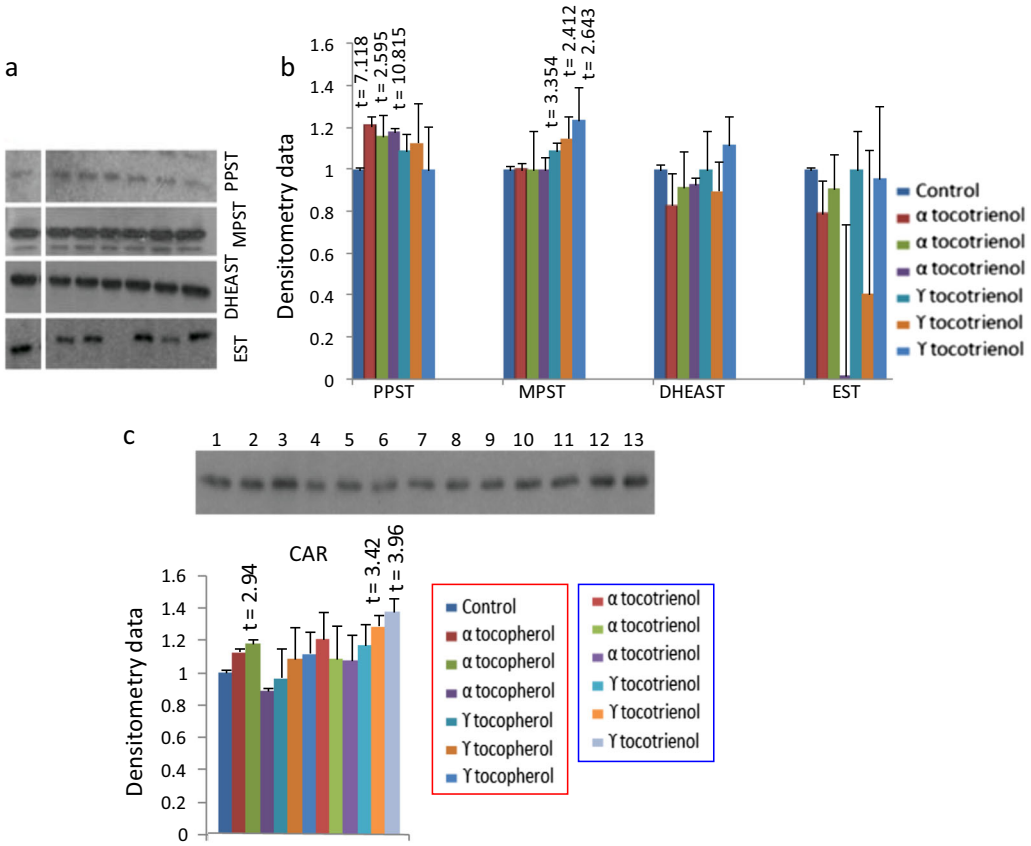

Fig. 4 Representative blot shows human SULTs expression in response to vitamin E (tocotrienol) exposure in HepG2 cells (**a**) and those desitometry analyses is plotted as bar plot (**b**) from multiple repeat experiments. PPST phenol catalyzing sulfotransferase, MPST monoamine catalyzing sulfotransferase, DHEAST dehydroepiandrosterone catalyzing sulfotransferase, EST estrogen catalyzing sulfotransferase. Lane distribution is as follows, 1—control, 2, 3, 4 are 1, 10, and 100 μM of α-tocotrienol, respectively, and 5, 6, 7 are γ-tocotrienol of similar sequence of dose, respectively. The nuclear constitutive androstane receptor (CAR) protein expression and its densitometry analysis are shown in fig. **c** Lane distribution is as follows, 1—control, 2, 3, 4 are 1, 10, and 100 μM of α-tocopherol, respectively, and 5, 6, 7 are γ-tocopherol, 8, 9, 10 are 1, 10, and 100 μM of α-tocotrienol, respectively and 11, 12, 13 γ-tocotrienol, of similar sequence of dose, respectively. Data are presented as mean ± SD of three independent experiments. Level of significance are calculated after comparing with corresponding control at df = 4. p values are; $p = 0.05$ when $t = 2.7764$, $p = 0.01$ when $t = 4.604$, $p = 0.001$ when $t = 8.61$

nuclear receptors like constitutive androstane receptor (CAR) and PXR have been demonstrated. In this study, α-tocopherol and γ-tocotrienol have been noticed to moderately induce the CAR (Fig. 4c). The densitometry data also demonstrates some inductions to be significant at the highest dose of vitamin treatment.

Discussion

In physiological condition, free radicals promote several biochemical functions. But at the higher concentration they manifest different adverse effects. In this situation those are nullified by a number of antioxidant molecules like reduced glutathione, vitamin C and vitamin E or enzymes i.c. catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx). Free-radical mediated redox factor or antioxidant mediated free radical-nullification influence intracellular redox environment. These events impact redox-active enzyme functions. SULTs are the group of enzymes those are modified by the oxidative stress-dependant intracellular redox changes [23]. Large number of evidences from our and other laboratories supports this hypothesis [24, 25].

Global consumption of vitamin E is highly significant and that helps to reduce stress, associated diseases, and aging processes. This vitamin is the most prescribed micronutrient. Its effects on phase II drug-metabolizing enzymes and especially on SULTs expression has great practical values. Effects of vitamin E deficiency on hepatic microsomal cytochrome P450 and phase II enzymes suggest the importance to study this vitamin. Report reveals that lower P450 level due to low vitamin consumption might influence drug or endobiotic metabolism [26]. Alpha-tocopherol induced modulation of several genes/proteins other than SULTs is reported [27]. Here we report on vitamin E induced moderate regulations of human SULTs.

Other antioxidant vitamin like ascorbate has been shown to diversely influence SULTs expressions [28]. Vitamin C influences some sulfation metabolism by the inhibition of sulfatase 2 indirectly favouring the sulfation of some biomolecules. Vitamin C induced sulfation metabolism is reported to be associated with its anti-inflammatory and anti-cytotoxic effects in hepatocellular carcinoma [29]. Similarly, antioxidant property of vitamin E influences free-radical metabolism which may control oxidative stress-dependant NFκB and Nrf-2 role on inflammation. Several other micronutrients like vitamin D and K demonstrate SULT modulating abilities. In our study, increase of MPST and DHEAST expression by vitamin E (γ-tocopherol) may favor the direct sulfation related metabolism (Fig. 3), similar effect noticed by indirect action of vitamin C. Vitamin D is reported to induce several CYP (cytochrome P450) groups like CYP24A1, CYP3A4, and some phase II SULTs (e.g., SULT2B1b, a DHEA-sulfotransferase), CYP3A4 and SULT2B1b [30]. It is suggested that variation in the SULT2A1 genes expression contributes to inter-individual differences in vitamin D homeostasis. Sulfate metabolites may serve as a reservoir of $25OHD_3$ in individuals [31]. It may be true in case of vitamin E also. Further studies are required in this regard. As shown in the current study, vitamin E induction of MPST and DHEAST in HepG2 cells may indicate possible influence on the sulfation metabolism of monoamine and dehydroepiandrosterone (Fig. 3). Steroid metabolism and signaling is reported to influence on the aging process of the individuals. Age protection effect and antioxidative-therapeutic efficacies of vitamin may attribute via steroid metabolism. Previous work has shown that vitamin K regulates brain sulfotransferase activity [32, 33]. Micronutrient effects on the gene expressions of drug-metabolizing enzymes have been reported. Especially, DHEA is regarded to be vitality and youth promoting hormone, so it's possible regulations by micronutrients is important. We found that tocotrienol also exerts induction effects of several SULTs in the human hepatocytes (Fig. 4b). We found that the expression of CAR moderately increased in the current study so it may be assumed that vitamin E induced SULTs up-regulations may be occurring via CAR regulations (Fig. 4c). Report reveals that mice in late life are less responsive to the hormonal and xenobiotic signaling for SULT2A1 induction via nuclear-receptor regulation [10]. This suggests that age dependant SULTs regulations and sulfation metabolism is physiologically important. Our earlier report revealed that retinoic acid induced aryl sulfotransferase IV (ASTIV, equivalent to PPST) in liver of female rats and sulfotransferase a (STa, equivalent to DHEAST) in male rats [11]. And some of the regulations were due to redox modifications of the catalytically active Cys in the enzymes. Figure 2 shows the presence of Cys in the enzymes and some of those are close to either PAPS or substrates. Report reveals that α-tocopherol supplementation to diabetic patients increased GSH level (9%, $p < 0.05$) and concomitantly decreased oxidative stress. This finding has implication in our investigation. Vit E-mediated change in cellular oxidative stress might modulate SULTs activities. More studies are necessary [34].

It can be concluded that vitamin E has variable effects on SULTs induction in human HepG2 cells. Tocopherol and tocotrienols and their different forms like α and γ have differential effects on SULTs. And depending on the isoform of the SULTs vitamin E has variable effects. Transcriptional or translational regulations of proteins need a physical interaction of drug to the nucleic acids. Modification of the proteins is also the result of a molecular interaction with a drug to the protein. The different derivative of the vitamin E possesses different structures, so the interaction pattern would be different. That is why variable effects are noticed in the current study. Vitamin E is an antioxidant and its different forms significantly and variably influences the cellular oxidative stress. So the final outcome of the antioxidative–oxidative events determines the fate of SULTs expressions/activities. Several of our previous experiments and present study strongly suggest that reducing environment can activate SULTs activity as well as protein and mRNA expression. Though the induction effects are moderate but long-term consumption of this vitamin may significant alter SULTs expression and related sulfation metabolism.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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