



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



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(-)Epicatechin Regulation of Hydroxysteroid Sulfotransferase STa (rSULT2A1) Expression in Female Rat Steroidogenic Tissues

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ABSTRACT

The aim of this study was to investigate (-) epicatechin regulation of rSULT2A1 expression in female rat liver, adrenal gland, ovary, and heart. Western blotting and reverse transcription-polymerase chain reaction (RT-PCR) determined protein and mRNA levels, respectively. (-) epicatechin treatment (7 days) significantly inhibited rSULT2A1 protein and mRNA expression in rat ovary and heart but significantly induced that in adrenal gland. No significant changes in rSULT2A1 expression were observed in female rat liver. These results suggest that (-) epicatechin may increase the sulfation of DHEA in the DHEA producing adrenal gland, which will increase the transport and stability of DHEA. While in the target tissues such as heart and ovary, decreased SULT2A1 expression caused by (-) epicatechin will increase the de-sulfation of DHEAS to DHEA to increase the biological activity of DHEA. This may explain the mechanisms for the beneficial effects of (-) epicatechin in heart and ovary. (-) Epicatechin does not affect the detoxification roles of SULT2A1 in liver.

Key words: Sulfation, gene regulation, adrenal gland, endocrine, biological activity, flavonoids

INTRODUCTION

Cytosolic sulfotransferases (SULTs) are one of the major families of phase II drug-metabolizing enzymes. They catalyze the sulfation of hydroxyl-containing compounds, including endogenous and exogenous molecules ranging from hormones, monoamine neurotransmitters and peptides to drugs and xenobiotics (Glatt and Mehl, 2004; Gamage *et al.*, 2006; Lindsay *et al.*, 2008; Runge-Morris and Kocarek, 2009). Hydroxysteroid SULTs are a subfamily of SULTs that play important roles in the regulation of the biological functions of hydroxysteroids and detoxification of xenobiotics (Fang *et al.*, 2007; Gulcan *et al.*, 2008). A major isoform of hydroxysteroid SULTs in rats is rSULT2A1 (STa), which is involved in the sulfation of the hydroxysteroids dehydroepiandrosterone (DHEA), androsterone and cortisol (Apak and Duffel, 2004; Sinclair *et al.*, 2006; Gulcan *et al.*, 2008; Huang *et al.*, 2010). DHEA is a steroid hormone synthesized from cholesterol and secreted by the adrenal glands and is a precursor of all major sex hormones: estrogen, progesterone and testosterone (Leowattana, 2001). DHEA is called the mother hormone, because it is the source that fuels the body's metabolic pathways (Regelson *et al.*, 1994). DHEA is sulfated into DHEA sulfate (the inactive form of DHEA) by hydroxysteroid SULTs. DHEA

sulfate is considered as a buffer and reservoir. Sulfated DHEA can circulate in the blood and reach steroidogenic tissues such as ovary and heart, where it is converted to biologically active DHEA by steroid sulfatases. The major SULT isoform responsible for sulfating DHEA is rSULT2A1; thus, rSULT2A1 plays important roles in regulating DHEA-DHEA sulfate balance.

Naturally occurring flavonoids represent a main class of secondary metabolites in plants. They are widely distributed in plants, vegetables, fruits and in beverages such as tea, coffee and wine (Es-Safi *et al.*, 2007; Li and Jiang, 2007; Li *et al.*, 2007; Roman, 2007). Flavonoids are also major functional components of many herbal drug preparations that have been used for thousands of years. These compounds are important health-enhancing food components and dietary supplements. Flavonoids have been referred to as nature's biological response modifiers, because of strong experimental evidence supporting their inherent ability to modify the body's reaction to allergens, viruses and carcinogens.

Various phytochemical compounds in foods and beverages have been widely investigated in recent years. Cocoa contains high amounts of flavonoids, specifically the flavanol (-)epicatechin (EC) (Fig. 1) (Roura *et al.*, 2005). According to recent studies, Panama's Kuna Indian living on islands had significantly lower rates of heart disease, stroke and cancer than Kuna Indians living on the mainland (Hollenberg *et al.*, 1997; McCullough *et al.*, 2006). It was believed that this is because the Kuna Indian living on islands are heavy cocoa consumers while Kuna Indians living on the mainland do not consume cocoa. The high amount of EC in the cocoa is believed to contribute to this benefit effect. EC has also been reported to exhibit a wide range of biological activities. For example, EC increases the production of endothelial nitric oxide synthase (eNOS)-dependent nitric oxide (NO) and endothelium-derived vasodilators and improves endothelial function (Steffen *et al.*, 2005; Schroeter *et al.*, 2006). In addition, EC inhibits arginase activity, thereby improving endothelium-dependent vasorelaxation (Schnorr *et al.*, 2008). EC also mimics insulin effectors in restoring acetylcholine esterase activity to normal levels in diabetic erythrocytes (Rizvi and Zaid, 2001) and has antioxidant effects against lipid peroxidation (Terao *et al.*, 1994).

Dietary flavonoids can affect the bioavailability of endogenous hormones by acting as substrates and/or inhibitors of human SULTs. EC is the most abundant flavonoid found in cocoa. Sulfation is the major metabolic pathway targeted by EC in the human liver and intestine (Vaidyanathan and Walle, 2002; Ung and Nagar, 2007). In addition, EC has been shown to inhibit SULT activities in liver cytosol (Harris *et al.*, 2004; Nishimuta *et al.*, 2007). To date, it is still

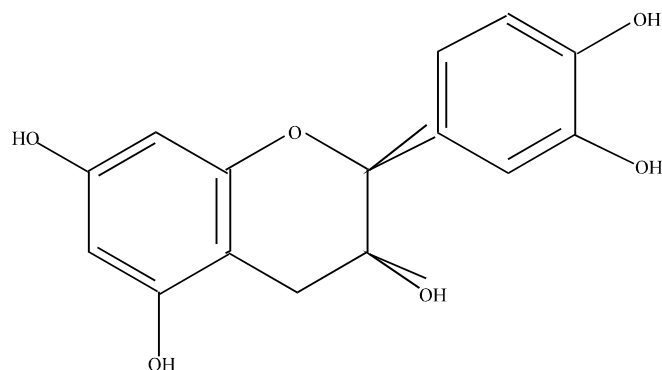


Fig. 1: Chemical structure of (-)epicatechin (cis--2-[3,4-Dihydroxyphenyl]-3,4-dihydro-2H-1-benzopyran-3,5,7-triol)

unknown whether EC affects SULT gene expression. Studies on cytosolic SULTs have mostly focused on detoxification tissues such as liver and intestine. Few studies have been done on SULTs in other tissues such as heart, ovary and endocrine glands like the adrenal gland. In the present study, we studied how EC treatment affects the gene expression of the hydroxysteroid SULT rSULT2A1 in female rat liver, adrenal gland, ovary and heart. To the best of our knowledge, this is the first study to examine EC-mediated gene regulation of rSULT2A1 in steroidogenic tissues.

MATERIALS AND METHODS

(-)-Epicatechin (cis-2- [3, 4-Dihydroxyphenyl] -3, 4-dihydro-2H-1-benzopyran-3, 5, 7-triol) was purchased from MP Biochemicals, Inc. SDS-polyacrylamide gel electrophoresis reagents and protein assay reagent were obtained from Bio-Rad (Hercules, CA). Adenosine 3'-phosphate, 5'-phosphosulfate (PAPS) was from Sigma Chemical Company. Western blot chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/Enhancer solutions) were purchased from Pierce Chemical (Rockford, IL). Nitrocellulose membranes (Immobilon-P; Millipore Corporation, Bedford, MA) used for the Western blot procedure was from Fisher Scientific Co. (Fair Lawn, NJ). Total RNA extraction kit (Tri reagent) was supplied by Molecular Research Center. SuperScript III Reverse transcriptase was from Invitrogen. GoTaq DNA polymerase, dNTP and random primers were from Promega. We purchased qPCR™ Mastermix Plus for SYBR Green I from Eurogentec. [1, 2, 6, 7-³H (N)] dehydroepiandrosterone was from NEN (Boston, MA). Antibodies against rat Sta (Chen *et al.*, 1995) were generously provided by Dr. Michael W. Duffel (Division of Medicinal and Natural Products Chemistry, College of Pharmacy, the University of Iowa, Iowa City, IA). This research project was carried out between the summer of 2008 and the summer of 2010.

Rat treatment: Female Sprague-Dawley rats (Harlan, Indianapolis, IN), 10 to 11 weeks old and 210 to 230 g body weight, total of 32 rats, were used for this study. Rats were housed in a temperature- and humidity-controlled room and supplied with rodent chow and water for at least one week before use. Rats were divided into four groups with four rats per group. EC was suspended in corn oil and three groups of female rats were oral fed by gavage 3, 12 and 48 mg kg day of EC for 7 days. The corresponding control rats received only the vehicle (corn oil). The animals were sacrificed 24 h after the last treatment. Adrenal glands, ovaries, hearts and livers were collected and frozen immediately in liquid nitrogen. Samples were stored at -80°C until use.

Rat tissue cytosol preparation: Adrenal gland, ovary, heart and liver homogenates were prepared with 50 mM Tris buffer (pH 7.4), containing 0.25 M sucrose, 3 mM 2-mercaptoethanol, 0.02% Tween 20 and 10 µg mL⁻¹ phenylmethylsulfonyl fluoride. All homogenates were centrifuged at 100,000 g for 1h at 4°C. Cytosols (supernatants) were collected and stored at -80°C for Western blotting and enzyme activity assay.

Extraction of total RNA and RT-PCR: Total RNA was extracted from the adrenal gland, ovary, heart and liver samples using a single-step isolation method and TRIzol reagent according to the manufacturer's instructions. Superscript III (Invitrogen) reverse transcriptase and 2 µg of total RNA were used to synthesize the first-strand cDNA; 0.5 µL of cDNA was subjected to RT-PCR amplification on a PTC-100™ Programmable Thermal Controller (MJ Research INC.) All of the primers were designed in our laboratory using Primer Express 3.0 software (Applied Biosystems,

Carlsbad, CA). We synthesized the 131 bp amplification product of rSULT2A1 using the following forward (FW) and reverse (RE) primers: FW, 5'-AATCTCATGGAGAAGGAACTGATTCT-3'; RE, 5'-GGCCATTTTCTCCTGAAACACT-3' (GenBank accession No. NM_012695). The specificity of all primers was tested using BLAST Open Reading Frame software (National Center for Biotechnology Information).

The PCR conditions were as follows: 95°C for 2 min, 32 cycles of 45 s at 95°C, 45 s at 60°C, 35 s at 72°C, followed by a final extension of 7 min at 72°C. Rat β -actin expression was analyzed as a control. We synthesized the 116 bp amplification product of rat β -actin from the same amount of template using the following primers: FW, 5'-AGGCCCTCTGAACCCTAAG-3'; RE, 5'-AGAGGCATACAGGGACAACACA-3'. The PCR conditions were the same as described above, except 26 cycles were performed.

Quantitative real-time PCR: Real-time PCR was performed using QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. Primers were the same as those used for RT-PCR, as described above. Real-time PCR was performed on an ABI PRISM 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Initially, regular PCR products were purified with GENECLAN Turbo (Qbiogene, Carlsbad, CA) for constructing standard curves (10 - 10^8 copies). A standard curve was plotted: threshold cycle (C_T) versus the logarithmic value of the gene copy number. The gene copy number of unknown samples was generated directly from the standard curve with Sequence Detector 1.7 software provided by the 7500 Fast Real-Time PCR system. Each sample was run in duplicate and each experiment was repeated three times. All gene copy numbers were normalized to rat β -actin mRNA.

Western blot analysis of rSULT2A1 protein expressed in rat adrenal gland, ovary, heart and liver tissues: Cytosol protein from adrenal gland, heart, ovary (40 μ g), or liver (5 μ g) were subjected to electrophoresis on 12% polyacrylamide gels (Novex, San Diego, CA) at 160V for about 60 min. Separated proteins were electro-transferred onto nitrocellulose membranes at 25 V overnight. Membranes were blocked in TBST (50mM Tris [pH 7.5], 150mM NaCl, 0.05% Tween20) containing 5% dried milk for 30 min on a shaker at room temperature. After blocking, membranes were incubated with rabbit anti-rSULT2A1 (1:2000) in TBST containing 5% dried milk overnight on a shaker at 4°C (or for 2 h at room temperature). After incubation, membranes were washed with TBST three times for 15 min each and incubated in secondary antibody (horseradish peroxidase-conjugated Immuno-Pure goat anti-rabbit IgG; H+L) at a dilution of 1:10000 in TBST containing 5% dried milk for 1h. The membranes were washed with TBST three times for 15 min each. Fluorescent bands were developed with 1ml of substrate containing the same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at room temperature for 5 min. Images of the bands were developed with a VersaDoc 5000 MP imaging system (BIO-RAD Laboratories, Inc.). Densitometric analyses were performed using Quantity One version 4.6.5 Analysis Software provided by the VersaDoc 5000 MP imaging system.

Statistical analysis: One-way ANOVA followed by the Dunnett's test was used to calculate the statistical significance of the difference between the control group means and EC treatment group means (Troendle, 1996). In all cases, $p < 0.05$ was considered significant and $p < 0.01$ was considered very significant. Data presented are means $\pm$ standard deviation (SD) of the data collected separately from four individual animals.

RESULTS

Differential expression of rSULT2A1 in female rat ovary, heart, adrenal gland and liver:

The SULT rSULT2A1 (STa) is a hydroxysteroid SULT whose expression is gender and age dependent. Real-time PCR was employed to determine the expression of rSULT2A1 mRNA in rat ovary, heart, adrenal gland and liver. Figure 2 shows that rSULT2A1 mRNA was highly expressed in female rat liver. In addition, rSULT2A1 expression was also detected in female rat ovary, heart and adrenal gland.

(-)Epicatechin downregulation of rSULT2A1 expression in rat ovary: The expression of rSULT2A1 protein in ovary was determined by Western blot, as shown in Fig. 3A. After 7 days of EC treatment, rSULT2A1 protein levels decreased, as shown by the decreased intensity of the rSULT2A1 protein band. Densitometry analysis revealed that protein content decreased by 70% ($p<0.001$) and 53% ($p<0.01$) after 12 and 48 mg kg day EC treatment, respectively. Quantitative real-time PCR demonstrated that EC inhibited rSULT2A1 mRNA expression in ovary and that mRNA copy number decreased by 71% ($p<0.01$) and 52% ($p<0.05$) after EC treatment at doses of 12 and 48 mg kg day, respectively (Fig. 3B). The Western blot results (Fig. 3A) were in good agreement with the real-time PCR results (Fig. 3B).

(-)Epicatechin downregulation of rSULT2A1 expression in rat heart: The expression of rSULT2A1 protein in heart was determined by Western blot, as shown in Fig. 4A. After 7 days of EC treatment, protein levels decreased, as shown by the decreased intensity of the rSULT2A1 protein band. Densitometry analysis revealed that protein content decreased by 85% ($p<0.001$) and 67% ($p<0.01$) after 12 and 48 mg kg day EC treatment, respectively. Quantitative real-time PCR also demonstrated that EC inhibited rSULT2A1 mRNA expression in heart (Fig. 4B). Moreover, mRNA copy number decreased by 50% ($p<0.05$), 74% ($p<0.01$) and 88% ($p<0.001$) after EC treatment at doses of 3, 12 and 48 mg kg day, respectively. The Western blot results were in good agreement with real-time PCR results.

(-)Epicatechin upregulation of rSULT2A1 expression in female rat adrenal gland: The expression of rSULT2A1 protein in adrenal gland was determined by Western blot, as shown in

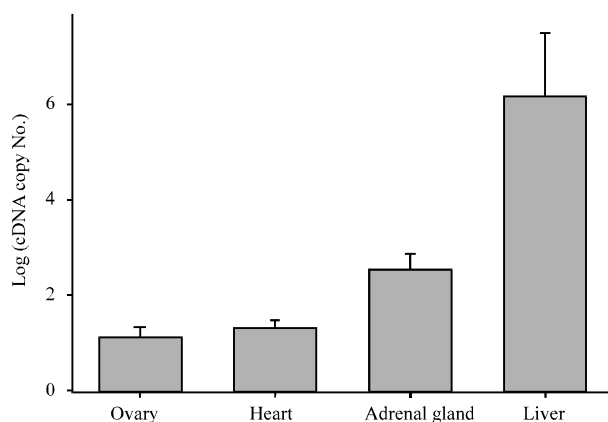


Fig. 2: Expression of rSULT2A1 mRNA in female rat liver, adrenal gland, ovary and heart. cDNA was synthesized from 2 μ g of total RNA and rSULT2A1 mRNA copy number was normalized to rat β -actin mRNA

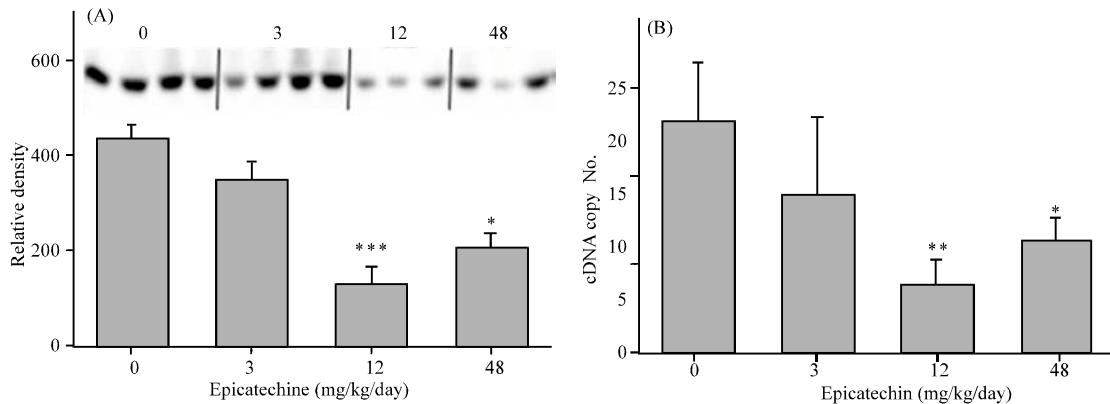


Fig. 3: Inhibition of rSULT2A1 by (-)epicatechin in rat ovary. Female rats were treated with different doses of EC for 7 days. (A) Representative Western blot and corresponding densitometry values. (B) Real-time PCR. cDNA was synthesized from 2 μ g of total RNA and rSULT2A1 mRNA copy number was normalized to rat β -actin mRNA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control

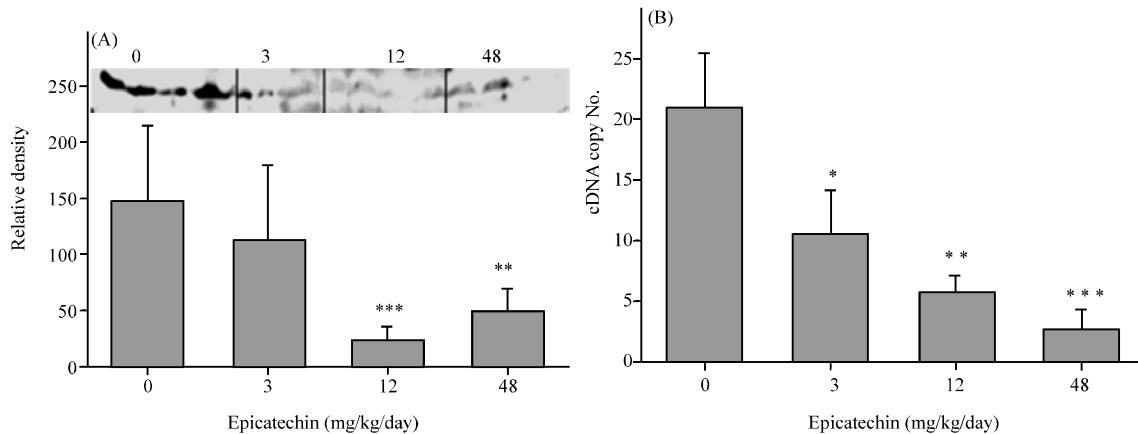


Fig. 4: Inhibition of rSULT2A1 by (-)epicatechin in female rat heart. Female rats were treated with different doses of EC for 7 days. (A) Representative Western blot and corresponding densitometry values. (B) Real-time PCR. cDNA was synthesized from 2 μ g of total RNA and rSULT2A1 mRNA copy number was normalized to rat β -actin mRNA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with control

Fig. 5A. After 7 days of EC treatment, rSULT2A1 protein levels increased, as shown by the increased intensity of the rSULT2A1 protein band. Densitometry analysis revealed that protein content increased by 6.0 fold ($p < 0.001$) and 9.7 fold ($p < 0.001$) after 12 and 48 mg kg day EC treatment, respectively. Quantitative real-time PCR demonstrated that rSULT2A1 mRNA expression in adrenal gland was also induced in a dose-dependent manner and that mRNA copy number increased by 2.2 fold ($p < 0.01$) and 3.4 fold ($p < 0.05$) after EC treatment at doses of 12 and 48 mg kg day, respectively (Fig. 5B). We also used traditional RT-PCR to determine rSULT2A1 mRNA expression in adrenal gland. Figure 5C shows that rSULT2A1 mRNA levels in adrenal gland

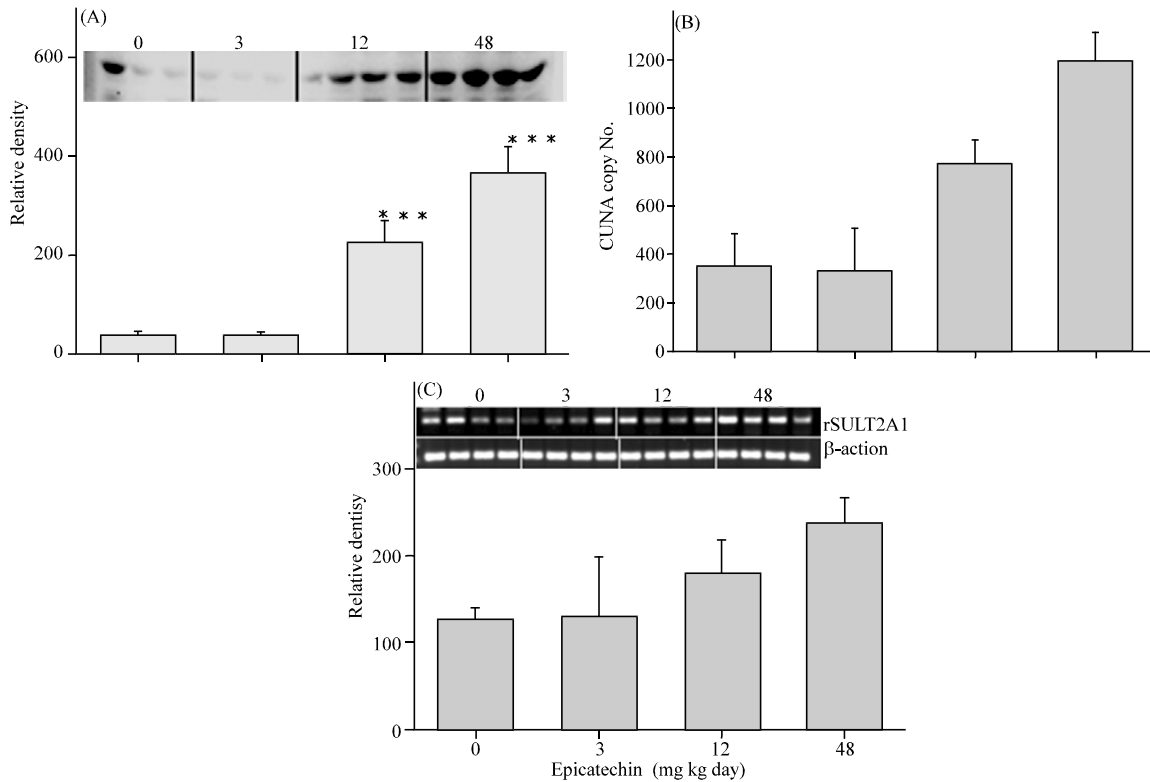


Fig. 5: Induction of rSULT2A1 by (-)-epicatechin in female rat adrenal gland. Female rats were treated with different doses of EC for 7 days. (A) Representative Western blot and corresponding densitometry values. (B) Real-time PCR. cDNA was synthesized from 2 μ g of total RNA and rSULT2A1 mRNA copy number was normalized to rat β -actin mRNA. (C) Traditional RT-PCR. Densitometry values corresponding to RT-PCR products are shown. * p <0.05, ** p <0.01, *** p <0.001 compared with control

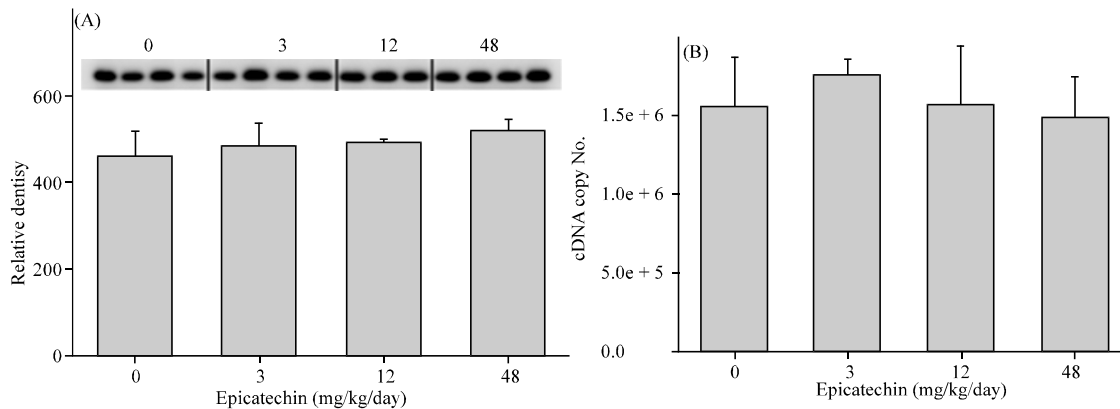


Fig. 6: Regulation of rSULT2A1 by (-)-epicatechin in female rat liver. Female rats were treated with different doses of EC for 7 days. (A) Representative Western blot and corresponding densitometry values. (B) Real-time PCR. cDNA was synthesized from 2 μ g of total RNA and rSULT2A1 mRNA copy number was normalized to rat β -actin mRNA

significantly increased in a dose-dependent manner after EC treatment. Densitometry analysis demonstrated that mRNA content increased by 1.4 fold and 1.9 fold ($p < 0.01$) after EC treatment at dose of 12 and 48 mg/kg/day, respectively.

rSULT2A1 expression in female rat liver was not affected by (-) epicatechin: Liver is the most important organ for biotransformation. SULTs are highly expressed in liver and rSULT2A1 especially is highly expressed in female rat liver. Western blot and real-time PCR analyses showed that EC treatment did not significantly affect rSULT2A1 expression in liver (Fig. 6A, B).

DISCUSSION

EC is a flavanol, a subgroup of flavonoids. EC is present in many plants. High quantities can be found in cocoa, tea and grapes (Vaidyanathan and Walle, 2002; Schuier *et al.*, 2005; Schroeter *et al.*, 2006; Roura *et al.*, 2007). Various studies have shown that EC plays important physiological roles. It reduces lipid peroxidation and inhibits platelet aggregation (Katiyar *et al.*, 1994; Terao *et al.*, 1994) induces peripheral vasodilation; improves endothelial function via a nitric oxide-dependent mechanism (Steffen *et al.*, 2005; Schroeter *et al.*, 2006) and decreases arginase-2 mRNA expression and arginase activity (Schnorr *et al.*, 2008). Especially, EC has been shown to have beneficial effects on cardiovascular health (Steffen *et al.*, 2005; Schroeter *et al.*, 2006; Yamazaki *et al.*, 2008). In the present investigation, we report the effects of EC on the gene expression of the hydroxysteroid SULT rSULT2A1 in female rat adrenal gland, ovary, heart and liver. To the best of our knowledge, this is the first report to describe SULT regulation in adrenal gland, ovary and heart.

Our results indicated that rSULT2A1 mRNA was differentially expressed in steroidogenic tissues. The expression of rSULT2A1 was highest in liver. The expression of rSULT2A1 was also detected in female rat ovary, heart and adrenal gland (Fig. 2). The regulation of rSULT2A1 expression by EC was also differential in these four steroidogenic tissues. EC inhibited rSULT2A1 expression both in female rat ovary and heart. However, in female rat adrenal gland, EC induced rSULT2A1 expression. In female rat liver, EC did not significantly affect the expression of rSULT2A1.

EC regulation of rSULT2A1 expression has physiological and clinical importance, as rSULT2A1 plays an important role in DHEA sulfation. DHEA is a steroid hormone synthesized from cholesterol and secreted by the adrenal glands. It is a very powerful precursor of major sex hormones: estrogen, progesterone and testosterone (Leowattana, 2001). Epidemiological studies have shown that low levels of DHEA correlate with a higher risk of cardiovascular disease (Feldman *et al.*, 2001). Thus, DHEA therapy has been suggested to be beneficial against coronary heart disease (Rabijewski and Zgliczynski, 2005). Indeed, DHEA and/or its metabolites exert cardioprotective actions through antihypertrophic effects (Nakamura *et al.*, 2004). Additionally, higher DHEA levels seem to positively affect endothelial cell signaling, which could have important implications for avoiding heart disease (Mohan and Benghuzzi, 1997; Varet *et al.*, 2004). In the present study, we found that EC inhibited rSULT2A1 expression in rat heart, suggesting that EC treatment may increase the levels of bioactive DHEA in the heart. Thus, EC inhibition of rSULT2A1 may have a beneficial impact on cardiovascular health.

We also found that EC inhibited rSULT2A1 expression in rat ovary. An ovary is an ovum-producing reproductive organ in female organisms. The ovaries have two functions: They produce eggs and female hormones, such as estrogen and progesterone, of which DHEA is a

precursor. DHEA treatment is known to increase oocyte and embryo numbers and fertilization rate (Barad and Gleicher, 2005, 2006). Genes encoding enzymes that regulate DHEA sulfonation are associated with DHEA sulfate levels in polycystic ovary syndrome (Goodarzi *et al.*, 2007). Because EC inhibits rSULT2A1 expression in rat ovary, it follows that EC also inhibits DHEA sulfonation in rat ovary. The latter would increase bioactive DHEA levels in the ovaries, which may have beneficial effect on ovarian function.

In the present study, EC did not significantly affect rSULT2A1 expression in rat liver, suggesting that EC does not affect the detoxification activity of SULT2A1 in liver.

EC regulation of rSULT2A1 may be mediated through certain nuclear receptors (Kim *et al.*, 2004; Seely *et al.*, 2005; Chen *et al.*, 2006; Fang *et al.*, 2007). EC can act as either an agonist or an antagonist of certain endogenous hormones that regulate rSULT2A1 expression *in vivo*. In different tissues, EC may induce rSULT2A1 through certain nuclear receptors or inhibit the expression of nuclear receptors that are regulated through endogenous hormones by competitively binding to these nuclear receptors. This will depend on endogenous hormone levels and nuclear receptor expression levels in different tissues. Hormone levels and nuclear receptor expression levels should differ across these tissues. This may explain why EC-mediated inhibition of rSULT2A1 expression was observed in ovary and heart, but EC-mediated induction of rSULT2A1 expression was observed in rat adrenal gland.

CONCLUSIONS

Present results demonstrated that the expression the major hydroxysteroid SULT, rSULT2A1, is significantly up-regulated by the dietary flavanol, EC, in the DHEA producing endocrine gland, adrenal gland. The increased SULT2A1 will increase the sulfation of DHEA; therefore increase its transport and stability. However, the expression of rSULT2A1 is significantly inhibited in heart and ovary. The decreased SULT2A1 will increase the de-sulfation of DHEA sulfate by sulfatases and increase the biological activities of DHEA in the targeted tissues. The results suggest the mechanisms for the beneficial effects of EC to heart and ovary. rSULT2A1 was not affected by EC in rat liver, suggesting that EC will not affect the detoxification roles of SULT2A1.

ACKNOWLEDGMENTS

This study was supported in part by National Institutes of Health (NIH) grant GM078606 (G.C.), American Cancer Society (ACS) grant RSG-07-028-01-CNE (G.C.), United States Department of Agriculture (USDA) grant 2006-35200-17137 (G.C.) and Oklahoma Center for the Advancement of Science and Technology (OCAST) grant HR05-015 (G.C.).

REFERENCES

- Apak, T.I. and M.W. Duffel, 2004. Interactions of the stereoisomers of alpha-hydroxytamoxifen with human hydroxysteroid sulfotransferase SULT2A1 and rat hydroxysteroid sulfotransferase STa. *Drug Metab. Dispos.*, 32: 1501-1508.
- Barad, D.H. and N. Gleicher, 2005. Increased oocyte production after treatment with dehydroepiandrosterone. *Fertil Steril*, 84: 756.e1-756.e3.
- Barad, D. and N. Gleicher, 2006. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. *Hum. Reprod.*, 21: 2845-2849.
- Chen, G., J. Baron and M.W. Duffel, 1995. Enzyme- and sex-specific differences in the intralobular localizations and distributions of aryl sulfotransferase IV (tyrosine-ester sulfotransferase) and alcohol (hydroxysteroid) sulfotransferase a in rat liver. *Drug Metab. Dispos.*, 23: 1346-1352.

- Chen, X., S. Maiti, J. Zhang and G. Chen, 2006. Nuclear receptor interactions in methotrexate induction of human dehydroepiandrosterone sulfotransferase (hSULT2A1). *J. Biochem. Mol. Toxicol.*, 20: 309-317.
- Es-Safi, N.E., S. Ghidouche and P.H. Ducrot, 2007. Flavonoids: Hemisynthesis, reactivity, characterization and free radical scavenging activity. *Molecules*, 12: 2228-2258.
- Fang, H.L., S.C. Strom, J.L. Falany, T.A. Kocarek and M. Runge-Morris, 2007. Positive and negative regulation of human hepatic hydroxysteroid sulfotransferase (SULT2A1) gene transcription by rifampicin: Roles of hepatocyte nuclear factor 4 α and pregnane X receptor. *J. Pharmacol. Exp. Ther.*, 323: 586-598.
- Feldman, H.A., C.B. Johannes, A.B. Araujo, B.A. Mohr, C. Longcope and J.B. McKinlay, 2001. Low dehydroepiandrosterone and ischemic heart disease in middle-aged men: Prospective results from the Massachusetts male aging study. *Am. J. Epidemiol.*, 153: 79-89.
- Gamage, N., A. Barnett, N. Hempel, R.G. Duggleby and K.F. Windmill *et al.*, 2006. Human sulfotransferases and their role in chemical metabolism. *Toxicol. Sci.*, 90: 5-22.
- Glatt, H. and W. Meinel, 2004. Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 369: 55-68.
- Goodarzi, M.O., H.J. Antoine and R. Azziz, 2007. Genes for enzymes regulating dehydroepiandrosterone sulfonation are associated with levels of dehydroepiandrosterone sulfate in polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, 92: 2659-2664.
- Gulcan, H.O., Y. Liu and M.W. Duffel, 2008. Pentachlorophenol and other chlorinated phenols are substrates for human hydroxysteroid sulfotransferase hSULT2A1. *Chem. Res. Toxicol.*, 21: 1503-1508.
- Harris, R.M., D.M. Wood, L. Bottomley, S. Blagg and C.J. Kirk *et al.*, 2004. Phytoestrogens are potent inhibitors of estrogen sulfation: Implications for breast cancer risk and treatment. *J. Clin. Endocrinol. Metab.*, 89: 1779-1787.
- Hollenberg, N.K., G. Martinez, M. McCullough, T. Meinking and D. Passan *et al.*, 1997. Aging, acculturation, salt intake and hypertension in the Kuna of Panama. *Hypertension*, 29: 171-176.
- Huang, J., S.P. Bathena, J. Tong, M. Roth, B. Hagenbuch and Y. Alnouti, 2010. Kinetic analysis of bile acid sulfation by stably expressed human sulfotransferase 2A1 (SULT2A1). *Xenobiotica*, 40: 184-194.
- Katiyar, S.K., R. Agarwal and H. Mukhtar, 1994. Inhibition of spontaneous and photo-enhanced lipid peroxidation and mouse epidermal microsomes by epicatechin derivatives from green tea. *Cancer Lett.*, 79: 61-66.
- Kim, M.S., J. Shigenaga, A. Moser, C. Grunfeld and K.R. Feingold, 2004. Suppression of DHEA sulfotransferase (Sult2A1) during the acute-phase response. *Am. J. Physiol. Endocrinol. Metab.*, 287: E731-E738.
- Leowattana, W., 2001. DHEA(S): The fountain of youth. *J. Med. Assoc. Thai.*, 84: S605-S612.
- Li, J. and Y. Jiang, 2007. Litchi flavonoids: Isolation, identification and biological activity. *Molecules*, 12: 745-758.
- Li, Y., H. Fang and W. Xu, 2007. Recent advance in the research of flavonoids as anticancer agents. *Mini. Rev. Med. Chem.*, 7: 663-678.
- Lindsay, J., L.L. Wang, Y. Li and S.F. Zhou, 2008. Structure, function and polymorphism of human cytosolic sulfotransferases. *Curr. Drug Metab.*, 9: 99-105.

- McCullough, M.L., K. Chevaux, L. Jackson, M. Preston and G. Martinez *et al.*, 2006. Hypertension, the Kuna and the epidemiology of flavanols. *J. Cardiovasc. Pharmacol.*, 47: S103-S109.
- Mohan, P.F. and H. Benghuzzi, 1997. Effect of dehydroepiandrosterone on endothelial cell proliferation. *Biomed. Sci. Instrum.*, 33: 550-555.
- Nakamura, S., M. Yoshimura, M. Nakayama, T. Ito and Y. Mizuno *et al.*, 2004. Possible association of heart failure status with synthetic balance between aldosterone and dehydroepiandrosterone in human heart. *Circulation*, 110: 1787-1793.
- Nishimuta, H., H. Ohtani, M. Tsujimoto, K. Ogura, A. Hiratsuka and Y. Sawada, 2007. Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. *Biopharm. Drug. Dispos.*, 28: 491-500.
- Rabijewski, M. and W. Zgliczynski, 2005. Positive effects of DHEA therapy on insulin resistance and lipids in men with angiographically verified coronary heart disease--preliminary study. *Endokrynol. Pol.*, 56: 904-910.
- Regelson, W., R. Loria and M. Kalimi, 1994. Dehydroepiandrosterone (DHEA): The mother steroid. I. Immunologic action. *Ann. N. Y. Acad. Sci.*, 719: 553-563.
- Rizvi, S.I. and M.A. Zaid, 2001. Insulin-like effect of (-)epicatechin on erythrocyte membrane acetylcholinesterase activity in type 2 diabetes mellitus. *Clin. Exp. Pharmacol. Physiol.*, 28: 776-778.
- Roman, G.C., 2007. Autism: Transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. *J. Neurol. Sci.*, 262: 15-26.
- Roura, E., C. Andres-Lacueva, O. Jauregui, E. Badia, R. Estruch, M. Izquierdo-Pulido and R.M. Lamuela-Raventos, 2005. Rapid liquid chromatography tandem mass spectrometry assay to quantify plasma (-)-epicatechin metabolites after ingestion of a standard portion of cocoa beverage in humans. *J. Agric. Food Chem.*, 53: 6190-6194.
- Roura, E., M.P. Almajano, M.L. Bilbao, C. Andres-Lacueva, R. Estruch and R.M. Lamuela-Raventos, 2007. Human urine: Epicatechin metabolites and antioxidant activity after cocoa beverage intake. *Free Radic. Res.*, 41: 943-949.
- Runge-Morris, M. and T.A. Kocarek, 2009. Regulation of sulfotransferase and UDP-glucuronosyltransferase gene expression by the PPARs. *PPAR Res.*, 2009: 728941-728941.
- Schnorr, O., T. Brossette, T.Y. Momma, P. Kleinbongard, C.L. Keen, H. Schroeter and H. Sies, 2008. Cocoa flavanols lower vascular arginase activity in human endothelial cells *in vitro* and in erythrocytes *in vivo*. *Arch. Biochem. Biophys.*, 476: 211-215.
- Schroeter, H., C. Heiss, J. Balzer, P. Kleinbongard and C.L. Keen *et al.*, 2006. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci. USA.*, 103: 1024-1029.
- Schuijter, M., H. Sies, B. Billek and H. Fischer, 2005. Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon Epithelia. *J. Nutr.*, 135: 2320-2325.
- Seely, J., T. Suzuki, B. Mayhew, H. Sasano and V. Giguere *et al.*, 2005. Transcriptional regulation of dehydroepiandrosterone sulfotransferase (SULT2A1) by estrogen-related receptor alpha. *Endocrinology*, 146: 3605-3613.
- Sinclair, P.A., W.J. Gilmore, Z. Lin, Y. Lou and E.J. Squires, 2006. Molecular cloning and regulation of porcine SULT2A1: Relationship between SULT2A1 expression and sulfoconjugation of androstenone. *J. Mol. Endocrinol.*, 36: 301-311.
- Steffen, Y., T. Schewe and H. Sies, 2005. Epicatechin protects endothelial cells against oxidized LDL and maintains NO synthase. *Biochem. Biophys. Res. Commun.*, 331: 1277-1283.

- Terao, J., M. Piskula and Q. Yao, 1994. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.*, 308: 278-284.
- Troendle, J.F., 1996. A permutational step-up method of testing multiple outcomes. *Biometrics*, 52: 846-859.
- Ung, D. and S. Nagar, 2007. Variable sulfation of dietary polyphenols by recombinant human sulfotransferase (SULT) 1A1 genetic variants and SULT1E1. *Drug. Metab. Dispos.*, 35: 740-746.
- Vaidyanathan, J.B. and T. Walle, 2002. Glucuronidation and sulfation of the tea flavonoid (-)-epicatechin by the human and rat enzymes. *Drug Metab. Dispos.*, 30: 897-903.
- Varet, J., L. Vincent, Y. Akwa, P. Mirshahi and A. Lahary *et al.*, 2004. Dose-dependent effect of dehydroepiandrosterone, but not of its sulphate ester, on angiogenesis. *Eur. J. Pharmacol.*, 502: 21-30.
- Yamazaki, K.G., D. Romero-Perez, M. Barraza-Hidalgo, M. Cruz and M. Rivas *et al.*, 2008. Short and long-term effects of (-)-epicatechin on myocardial ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ Physiol.*, 295: 761-767.