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Sexual dimorphisms in the immune system of catechol-*O*-methyltransferase knockout mice

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ABSTRACT

The enzyme catechol-*O*-methyltransferase (COMT) is part of the metabolic pathway of 17β-estradiol, converting 2-hydroxyestradiol to 2-methoxyestradiol. We recently showed that administration of the COMT product 2-methoxyestradiol has anti-inflammatory and anti-osteoporotic effects. We have now investigated whether COMT affects the immune system, by immunologically phenotyping COMT deficient ($COMT^{-/-}$) mice.

Immunoglobulin production, T lymphocyte proliferation, NK cell cytotoxicity and oxygen radical production were assessed.

In male $COMT^{-/-}$-mice, the total number of T-, and B-lymphocytes from spleen increased but the T-cell proliferative response decreased. The NK cell population shifted toward less mature cells, leaving cytotoxic capacity unaffected. In $COMT^{-/-}$-females, a higher frequency of neutrophils was found but the oxygen radical production was unaltered.

In conclusion, only minor changes of the immune system were seen in COMT deficient mice, and the changes were usually seen in males. This study provides clues into how COMT activity, and hence gender differences, affects the immune system.

Introduction

It is well established that estrogens affect the development and regulation of the immune system (Straub 2007; Islander et al. 2011). The differentiation of T-, and B cells, as well as T-cell dependent inflammation, decreases by estrogen, while the immunoglobulin production increases (Marotti et al. 1984; Carlsten et al. 1989; Erlandsson et al. 2003, 2002). We and others have previously shown that also the cytotoxic capacity of natural killer (NK) cells decrease after estrogen treatment (Nilsson and Carlsten 1994; Hanna and Schneider 1983).

The most potent estrogen, 17β-estradiol (E2), is extensively metabolized by different enzymes. E2 is converted into 2-methoxyestradiol (2me2) *via* 2-hydroxyestradiol, and requires the enzyme catechol-*O*-methyltransferase (COMT; see Fig. 1; Martucci and Fishman 1993; Zhu et al. 1996; Zhu and Conney 1998). 2me2 has a 500-fold and 3200-fold lower affinity than estradiol for ERα and ERβ, respectively (Lavallee et al. 2002). The products of COMT are conjugated estrogens that have intrinsic effects (Schmidt et al. 2009). These conjugated estrogens can be demethylated by enzymes of the CYP450 system (Zhu et al. 1996; Dawling et al. 2001, 2003). The demethylation of products could have implications regarding the regulation of COMT activity, and be a source of parent estrogens (Zhu et al. 1996; Zhu and Conney 1998; Dawling et al. 2003). COMT is found in most mammalian tissues with the highest activity in the liver, where studies on COMT activity have shown sex differences (Mannisto and Kaakkola 1999; Tenorio-Laranga et al. 2009). COMT has been extensively studied in the field of neurology, as the enzyme is crucial in neurodegenerative disorders (Gogos et al. 1998; Bonifacio et al. 2007; Calati et al. 2011). In addition, mice with a homozygous deletion of COMT ($COMT^{-/-}$)

Abbreviations: 2me2, 2-methoxyestradiol; CL, chemo luminescence; COMT, catechol-*O*-methyltransferase; conA, concanavilin A; E2, 17β-estradiol; Ig, immunoglobulin; Mcpm, million counts per minute; NK cell, natural killer cell; ROS, reactive oxygen species; TNFα, tumor necrosis factor alpha.

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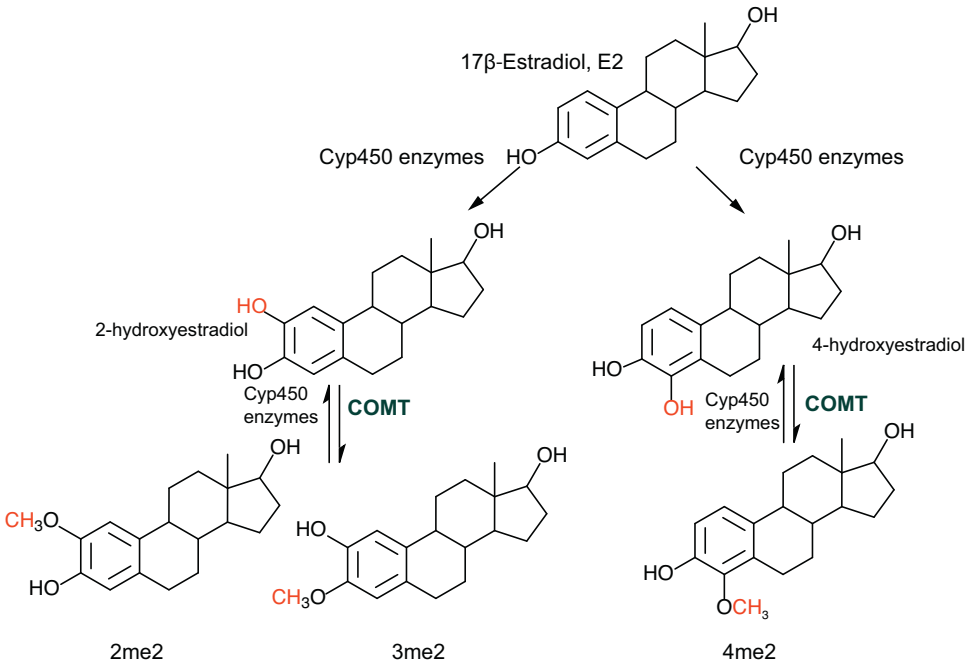

Fig. 1. Metabolism of 17β-estradiol by COMT. 17β-Estradiol is extensively metabolized by different enzymes. Many different isoforms of cytochrome P450 enzymes contribute to the hydroxylation of estradiol, also depending on tissue. These 2-hydroxylation and 4-hydroxylation metabolites can further be metabolized by the enzyme catechol-*O*-methyltransferase (COMT) into 2-methoxyestradiol (2me2), 3-methoxyestradiol (3me2) and 4-methoxyestradiol (4me2), as indicated. The reversal of the COMT methylation has been shown previously, catalyzed by the CYP450 enzyme system, where the best candidates are CYP1A1 and 1B1 (Zacharia et al. 2003; Dubey et al. 2003; Crooke et al. 2006; Schmidt et al. 2009; Martucci and Fishman 1993; Zhu et al. 1996; Parl et al. 2009; Dawling et al. 2001, 2003).

develop preeclampsia, a condition that involves the inflammatory system (Kanasaki et al. 2008). When these mice were reconstituted 2me2, the preeclampsia-like symptoms were reversed, and a full term pregnancy could be completed.

We and others have shown that 2me2 is an effective prophylactic (Josefsson and Tarkowski 1997) and therapeutic anti-inflammatory agent in experimental models of rheumatoid arthritis, with positive results on both arthritis and bone mineral density (Stubelius et al. 2011; Brahn et al. 2008; Issekutz and Sapru 2008). This indicates a role for COMT and 2me2 in the regulation and maintenance of the immune system.

In this study, we have investigated how the development and function of the immune system is affected in COMT$^{-/-}$-mice. Women have a higher incidence of autoimmune diseases than men (Whitacre 2001; Adrie et al. 2007). Calls for considerations of sex differences in both human and experimental trials have arisen in biomedical research (Ballantyne and Rogers 2011; Kim et al. 2010; Check Hayden 2010). In this study, we have investigated the development and function of the immune system, and differences between males and females, in COMT$^{-/-}$-mice.

Material and methods

Animal procedures

The generation of catechol-*O*-methyltransferase-deficient mice has been described previously (Gogos et al. 1998). The mutated COMT-allele was introduced into a mixed 129Sv/C57BL/6J genetic background and the mice were backcrossed > 10 generations to a C57BL/6J background. All mice were housed in a temperature- and humidity-controlled room with a 06:00–18:00 h light cycle and consumed a soy-free diet (R70, Lantmännen, Stockholm, Sweden) and tap water *ad libitum*. COMT disrupted homozygous mice (COMT$^{-/-}$) and their wild-type littermates, which were COMT+/+ (WT), were obtained by breeding heterozygous males and females. The Ethics Committee on Animal Care and Use in Gothenburg approved all procedures.

Genotyping

Genomic DNA was isolated from tail biopsies as previously described (Truett et al. 2000). For genotyping, a PCR method was employed using 5′-ACC ATG GAG ATT AAC CCT GAC TAC G-3′ (sense) and 5′-GTG TGT CTG GAA GGT AGC GGT C-3′ (antisense) primer set to detect COMT gene (*comt*) allele, and 5′-CAT TCT GCA CGC TTC AAA AG-3′ (sense) and 5′-TGT CTG TTG TGC CCA GTC AT-3′ (antisense) primer set to detect the PGK-Neomycin gene (*neo*) cassette that replaces exons 2–4 of the COMT gene. A 500 bp (COMT) and a 170 bp (neo) fragment were generated using ReddyPCRMix (ABgene®, Nucleic Acid Amplification, Epsom, United Kingdom) in the following thermal cycles; an initial temperature at 95 °C for 3 min and 35 cycles consisting of 95 °C for 1 min, annealing temperature of 55 °C for 30 s, and expansion at 72 °C for 1 min with a final extension at 72 °C for 5 min. The amplified DNA fragments were visualized using ethidium bromide staining under UV light after electrophoresis in 1% agarose gel.

Tissue collection

Mice were terminated at the age of 10–12 weeks. At the time of termination, mice were anesthetized with isoflurane inhalation (Baxter Medical AB, Kista, Sweden) or ketamine (PfizerAB, Täby, Sweden) and medetomidine injection (OrionPharma, Espoo, Finland), bled, and killed by cervical dislocation. Thymus, liver, spleen, uterus and seminal vesicles were weighed, and then thymus, spleen and whole bone were put in PBS. The liver was put into RPMI 1640 (without phenol red, PAA Laboratories GmbH, Pashing, Austria) after being perfused with saline.