Catecholoestrogens: possible role in systemic lupus erythematosus

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It is well established that risk of developing SLE is higher among women compared with men but only very little is understood about the underlying mechanisms. Oestrogen and their catechol metabolites seem to play an important role in SLE but the exact patho-aetiology remains elusive. The evidence concerning the possibility of catecholoestrogens (CEs) in the development of SLE are very limited and preliminary. The possible mechanism involves quinone–semiquinone redox cycling of CEs to generate the free radical that can cause DNA damage. This would probably alter its immunogenicity leading to the induction and elevated levels of SLE autoantibodies cross-reacting with native DNA. The data demonstrate the possible role of CE in presenting unique neo-epitopes that might form one of the factors in induction of SLE autoantibodies. However, the role of oestrogen in immune modulation cannot be ruled out as a mediator of various immune-related diseases.

Key words: Catecholoestrogen, Systemic lupus erythematosus, Autoantibodies, Reactive oxygen species, Catecholoestrogen-modified DNA.

Introduction

Sex hormones play an important role in the genesis of autoimmunity [1]. These hormones (oestrogens and their metabolites) are presumed to contribute to sexual dimorphism in the immune system. The presence or absence of oestrogen is implicated in the aetiology and pathogenesis of multiple diseases including breast and reproductive cancers, osteoporosis, coronary heart disease and systemic lupus erythematosus (SLE). SLE is a complex, chronic autoimmune disorder that is aetiologically multifactorial with both genetic and environmental factors contributing to disease initiation and progression [2]. The principal oestrogens in women (oestradiol and oestrone) undergo oxidative metabolism through hydroxylation at various sites, including 2- and 4-hydroxylation, leading to the formation of catecholoestrogen (CE, Fig. 1) [3, 4]. Studies have shown that constitutive and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-inducible P450 isozymes (P450 1A1/1A2 and P450 1B1), selectively catalyse hydroxylation at the 2- and 4-positions of oestrone and 17 β-oestradiol (E2) [5, 6]. 2-Hydroxylation is the major oxidative pathway, catalysed mainly by CYP 1A2 in the liver [7] and by CYP 1A1 in endometrium [8]. 4-Hydroxyoestrogen/oestradiol was found to be carcinogenic in the male Syrian golden hamster kidney tumour model, whereas 2-hydroxylated metabolites were without activity [9]. Previous study has shown that 4-hydroxyoestradiol (4-OHE2) induced uterine tumours in 66% of CD-1 mice, whereas mice treated with 2-hydroxyoestradiol or 17 β-oestradiol had much lower uterine tumour incidence [10]. The possible mechanism involves α-methylation of 2-hydroxyoestradiol to 2-methoxyoestradiol, which is a potent inhibitor of tumour cell proliferation and has anti-angiogenic effects [11]. Animal model produced a contradictory result with the ACI rat model for mammary carcinogenesis [12]. In contrast, DNA adducts of CE quinone have been detected in the mammary gland of ACI rats treated with 4-OHE2 or its quinone [13]. In females, significantly higher concentration of glutathione (GSH) conjugates resulting from reaction of GSH with the 4-OHE2/E2-quinones were detected in the non-tumour tissue from females with breast cancer as compared with controls [14]. In addition, oestrogen 4-hydroxylase level had a higher expression in the breast tissue of women with breast cancer as compared with the expression of protease enzyme [15]. Previous study showed a link between genetic polymorphism in oestrogen 4-hydroxylase and a risk for developing breast cancer [16]. Microsomes obtained from tumour tissues predominantly catalysed 4-hydroxylation, whereas those from normal tissues comparable oestradiol 2- and 4-hydroxylase activities were observed [17]. As previously described, oestrogen may be oxidized by hepatic cytochrome P450 enzyme (mainly CYP 1A1 and CYP 1B1) to hydroxy CEs and further oxidized to the semiquinone and quinone form [18]. The quinone formed from 4-hydroxyoestrogen has half-life of 12 min as compared with the short life of 2-OHE2 (t½ = 47s) [19], whereas, the quinone formed from 4-hydroxyequilenin (4-OHEN) is much more stable (t½ = 2.3h) than the endogenous CE [20]. It has been observed that adjacent aromatic ring stabilizes 4-OHEN-quinone through extended π-conjugation. In view of this, it has been demonstrated that the catechol metabolite of benzo[a]pyrene rapidly undergoes air oxidation to yield a very stable quinone, benzo[a]pyrene-7,8-dione [21].

A role for oestrogen in the pathogenesis of SLE has been suspected for many years but the exact patho-aetiology remains elusive. We herein review information pertaining to the possible role of CE in the pathogenesis of SLE. The central hypothesis of this review is that CEs formed in different tissue undergo oxidative metabolism to produce reactive oxygen species (ROS), which could cause damage in DNA. This would probably alter its immunogenicity leading to the induction and elevated levels of SLE autoantibodies cross-reacting with native DNA. In addition, our data strongly suggest that CE appears to play an important role in antigen-driven induction of SLE autoantibodies [22, 23].

CE and DNA damage

Studies of oestrogen metabolism, formation of DNA adducts, carcinogenicity, cell transformation and mutagenicity have led to the hypothesis that reaction of certain oestrogen metabolites, predominantly CE-3,4-quinone, with DNA can generate the critical mutations initiating different types of cancer [3, 13, 24]. They can undergo redox cycling with the semi-quinone...
radical, generating superoxide radicals mediated through P450 reductase [3]. The oestrogens are biochemically interconvertable in different form by enzyme 17-β-oestradiol dehydrogenase. These oestrogens are metabolized via two major pathways, i.e. formation of CEs and to a lesser extent, 16-α-hydroxylation. The catechols formed are the 2- and 4-hydroxylated oestrogens (Fig. 2) [25]. Generally, these two catecholestrogens can be inactivated by the enzyme catechol-α-methyltransferase (COMT) through α-methylation [26]. Other possible mechanism of inactivation includes the conjugation of CE by glucuronidation and sulphation. Excessive production of 4-hydroxylated metabolites caused insufficient production of methyl, glucuronide or sulphate conjugate which in turn results in CE toxicity in cell and consequently competitive catalytic oxidation to semiquinone (CE–SQ) and quinone (CE–Q). CE–SQ and CE–Q may conjugate with GSH, catalyse by S-transferase. If this inactivating process is incomplete, CE-Q may react with DNA to form stable and de-purinating adduct [13].

Various free radical toxicities have been reported in hamsters treated with E2 including DNA single-strand breaks [27], 8-oxo-2′-deoxyguanosine (dG) formation [28] and chromosomal abnormalities [29]. Recently, it has also been shown that 4-hydroxyoestradiol also induces oxidative stress and apoptosis in human mammary epithelial cells (MCF-10A) [30]. We have shown
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that 4-OHE2 is also capable of causing DNA single-strand breaks and oxidative damage to DNA bases *in vitro* [31, 32]. Finally, a recent study evaluated the potential of HRT to induce DNA damage in peripheral blood leucocytes of post-menopausal women using the comet assay [33]. Our results demonstrate that CE leads to the production of patent ROS, capable of causing DNA damage, thus playing an important role in carcinogenesis [31].

In order to see the genotoxic effect of CE-Q, they were reacted with the nucleosides 2′-deoxyguanosine (dG) and the nucleobase adenine [34]. The major DNA adducts produced from 4-hydroxyestradiol-o-quinone are depurinating N 7-guanine and N 3-adénine adducts resulting from 1,4-Michail addition both *in vitro* and *in vivo* [35, 36]. It is interesting to note that N 3-adénine adduct is likely to induce mutation since this adduct takes hours to hydrolyse [37]. Considerably more rapid isomerization of 2-hydroxyestraadiol-o-quinone to corresponding quinone methides (QM) results in 1,6-Michael addition products with exocyclic amino groups of adénine and guanine [38]. A depurinating N 3-adénine adduct of 2-hydroxyestraadiol QM has been recently reported in reaction with adénine and guanine [36]. The reaction of the CE 3,4-Q with dG at the N-7 position destabilizes the glycosidic bond and results in loss of the deoxyribose moiety. When the adduct is formed, it is released from the DNA by spontaneous depurination. It has been observed that reaction of E2-3-4-Q with dA produced no adducts, while reaction of E2-3-4-Q with Ade resulted in the formation of 4-OHE2-1-N3 Ade [34]. This adduct was formed only with Ade because in dA the adjacent deoxyribose bound to N-9 impedes the approach of the electrophile E2-3-4-Q to N-3 [39]. This interference is not present in DNA, as observed by formation of PAH-N3 Ade adducts, which are rapidly lost from the DNA by depurination [39, 40]. Oligonucleotides containing site-specific adducts transfected in simian kidney cells has been used to see mutagenic properties of 2-hydroxyestraadiol derived stable DNA adducts where G→T and A→T mutations were observed [41]. It is interesting to note that stable DNA adducts have been detected by 32P-postlabelling in Syrian hamster embryo cells treated with oestraadiol and its catechol metabolites [42]. The order in which the oestraadiol and its catechol metabolites can be categorized according to the DNA adduct formation was 4-OHE2 > 2-OHE1 > E2.

Finally, mutagenic adducts of CE corresponding to alklylation of guanine have been detected in human breast tumour tissue [43]. As mentioned above, oestrogens are metabolized by cytochrome P450 enzymes and form hydroxylated products [3]. The main metabolites of E2 include 2-, 4- and 16α-hydroxyestraadiol [3, 44]. The 2- and 4-hydroxylated catechols contain the hydroxyl groups, which predisposes them to further oxidation. Both can be oxidized to semiquinones and quinones with formation of superoxide anion radicals (O2−) [45]. These O2− readily dismutated to H2O2 either spontaneously or even faster when catalysed by superoxide dismutase. H2O2 is neutral but it may result in the formation of hydroxyl radicals [46]. However, as a neutral molecule, H2O2 can readily cross the cellular and nuclear membrane and reach DNA where it can cause oxidation of bases [47]. COMT prevent oxidation of CE to SQ by methylating 2- or 4-hydroxyl group. Moreover, 4-hydroxyl group of CE is not as readily methylated as is the 2-hydroxyl group, which can cause predominance of 4-OHE2 in redox cycling and inactivation of 2-OHE2 by methylation [48].

**CE and its immunology**

Sex hormones are presumed to contribute to sexual dimorphism in the immune system. These are implicated in immune response that can mediate humoral response [49]. In fact, at physiological doses, oestradiol induces IL-1α, a cytokine that can initiate a cascade of other cytokines, chemotactic and growth factors [50]. In addition, oestradiol also inhibits IL-1α-induced IL-6 production, which not only results in increased human epithelial cell proliferation (a process important in tumour growth) but also inhibits the activity of natural killer cells, thus allowing tumour growth [51]. Oestradiol also mediates macrophage proliferation and decreases cell differentiation [50].

Despite a large number of reports that may explain the role of oestrogen (or oestradiol) in modulation of immune response [49–51], the role of CE in the immune response is lacking. In view of this, we have in our earlier report showed that the CE-modified DNA (CE-DNA) was highly immunogenic (>1:25 600) in experimental animals [31]. These antibodies were effectively used as a probe for detecting oxidative lesions in human genomic DNA as well as for the estimation of 8-hydroxy-2′-deoxyguanosine (8-OHG) level in the urine of cancer patients [31]. In competition ELISA, these antibodies showed strong preference for the CE-DNA (immunogen) causing 92% inhibition in antibody binding at 20μg/ml of the immunogen (competitor) concentration. Fifty percent inhibition was observed at 3.75μg/ml of the immunogen. In addition, the antibodies also showed a high degree of binding towards CE-modified forms of guanine, thymine, human and calf thymus DNA, ROS-DNA, 8-OHG, etc. It is important to note that CE-DNA was not only recognized by cancer autoantibodies [31] but also by anti-DNA autoantibodies in SLE [22, 23]. Data from our laboratory suggest that CE are likely to contribute to the generation of antibodies which may directly or indirectly affect the immune system.

Oestrogen is metabolized into several different post-oestrogen hormones, namely 2-hydroxy, 4-hydroxy and 16-hydroxyestrogens [3]. Research has shown 4-hydroxy and 16-hydroxyestrogen to be powerful growth and inflammation promoters, especially in oestrogen-sensitive tissues such as the uterus, cervix and prostate [52]. On the other hand, 2-hydroxyestrogens have shown to be powerfully protective of those same tissues helping to prevent cancer and elevated prostate specific antigen (PSA) from prostate tissue [53].

Oestrogen causes macrophage proliferation and activation and in turn, macrophages produce oestrogen, which may act on other phagocytic cells. On stimulation, macrophages produce oxidants (such as O2− and H2O2) by a reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyse reduced of molecular oxygen. In addition, macrophages also synthesized inducible nitric oxide synthase and mediate production of nitric oxide (NO). O2− and NO may rapidly interact, with the generation of peroxynitrite, a much more potent oxidant [54]. Hence, macrophage activation may lead to ROS and reactive nitrogen species (RNS) production, which may cause DNA base hydroxylation, oxidation, nitration and deamination [55, 56]. Oestrogen not only causes macrophage proliferation and activation but also affects the function of PMNs (polymorphonuclear leucocytes, neutrophils and granulocytes), which is also responsible for the production of ROS on their stimulation. PMNs express myeloperoxidase, an enzyme that catalyses oxidation of chloride ions by H2O2 to hypo-chlorite/hypochlorous acid (HOCl/OCl−) [47]. This reaction is catalysed by myeloperoxidase released from PMNs during activation process. Oestrogens and some of their metabolites may induce myeloperoxidase release from the resting (inactivated) cells and stimulate generation of oxidants in the absence of pathogens [56]. It is interesting to note that 2-hydroxylated oestrogens act as powerful inhibitors of PMNs activity, thereby one of the protective properties of the 2-hydroxylated CE. Therefore, oestrogens affect inflammatory response and in turn, their activities are affected by the inflammation products.

**SLE**

Female hormones promote humoral autoimmunity. Sex hormone expression is different in patients with SLE than in healthy
controls [58]. High concentration of biologically potent oestrogens cause SLE patients to circulate more self-reactive lymphocytes that bypassed development deletion. SLE is a multisystem autoimmune disease characterized by production of autoantibodies against many different autoantigens, typically including nucleic acid and associated protein [59]. Significant health consequences include renal failure, vasculitis, thrombosis, seizures and other neurological complications [60]. The disease activity also considered as a major factor associated with menstrual cycle disorders in the patients [61]. Almost all autoimmune diseases are more common in women, but the female predominance in SLE is particularly strong. Of the patients >85% with SLE are women [62]. The central immunological disturbance in patients with SLE is autoantibody production. These are directed at several self-molecules found in nucleus, cytoplasm and cell surface [63]. In addition, autoantibodies are also directed against platelets, lymphocytes, polysaccharides [64], phospholipids [65], cell membrane structure [66] and nucleoproteins (DNA-histone, nuclear ribonucleoproteins like Sm and Mo, and cytoplasmic ribonucleoproteins) [67]. The most remarkable feature of anti-DNA antibodies is their association with glomerulonephritis. Anti-DNA antibodies can be isolated in an enriched form from glomerular eluates of patients with active lupus nephritis and these antibodies can induce nephritis in normal and immunodeficient mice [68]. Anti-DNA antibodies differ in their properties, including isotype, ability to fix complement and capacity to bind to the glomeruli causing pathogenicity [69]. Only certain types of anti-DNA antibodies are pathogenic [70]. First, clinical observation in most patients indicates that active nephritis is associated with raised anti-DNA antibody titres and reduced total haemolytic complement values [71]. Secondly, anti-DNA antibodies show preferential deposition in the kidneys, suggesting that DNA-anti-DNA antibody immune complexes are the main mediators of inflammation [72]. In addition, antibodies against CE-modified DNA have been detected in SLE patients [22, 23].

SLE is characterized by a myriad of immune system aberrations that involve B cells, T cells and cells of the monocytes lineage, resulting in polyclonal B-cell activation, increased number of antibody producing cells, hypergammaglobulinemia, autoantibody production and immune complex formation [59]. The activation of B- and T cells requires stimulation by specific antigens. Chemicals such as pristine, bacterial DNA and cell wall phospholipids and viral antigens can induce anti-DNA antibody production in mice [70]. Moreover, self-antigens, such as DNA-protein and RNA-protein complexes may induce autoantibody production [73]. B-cell activation is abnormal in patients with SLE. The number of B cells at all stages of activation is increased in the peripheral blood of patients with SLE [74]. These B-cell abnormalities can preclude the development of SLE. Activated lupus B cells have higher intracytoplasmic calcium responses than controls [75]. There is also evidence that B cells in patients with SLE are more sensitive to the stimulatory effects of cytokines such as IL-6 than non-SLE B cells [76]. Moreover, the phenomenon of epitope spreading has been demonstrated in both human and murine SLE [77]. Abnormalities in T-cell function are also evident in patients with SLE. The total number of peripheral blood T cells is usually reduced, probably because of the effects of anti-lymphocyte antibodies [78]. There is a skewing of T-cell function towards B cell help, leading to enhanced antibody production [76]. Experiments have shown that the early events of T-cell activation are defective in patients with SLE compared with controls [79]. Down-regulation by excessive Th 2 cytokines, defective interaction between APCs and T cells, the suppressive effects of CD8+ T cells and natural killer (NK) cells, the presence of IL-2 inhibitors and the down-regulation of IL-2 receptor are possible mechanisms [80].

Many cytokines have been implicated in regulating disease activity and in the involvement of different organs in patients with SLE [81]. Lupus T cells are less responsive to IL-2 stimulation than normal T cells [82]. However, the expression of IL-2 in freshly prepared SLE peripheral blood mononuclear cells (PBMCs) was increased compared with control PBMCs [83]. Lupus T cells are capable of producing normal amount of IL-2 in response to optimal stimulation with phytohaemagglutinin combined with phorbol esters or with anti-CD28 antibodies [84]. IL-10 played an important role in the pathogenesis of SLE. Earlier studies have shown that spontaneous production of IL-10 from SLE peripheral blood B cells and monocytes is significantly higher than controls [85]. The expression of IL-10 transcripts was significantly increased in the non-T-cell population of PBMCs from patients with SLE compared with controls [86]. Moreover, serum IL-10 concentration is higher in patients with SLE than in controls and is correlated with clinical and serological disease activity and anti-DNA antibody titres [87]. Similarly, IL-12 production was found to be impaired in stimulated PBMCs from patients with SLE compared with matched controls [88]. IL-12 promotes cell-mediated immune responses but exerts some inhibitory activities on humoral responses [89]. The results from these studies suggest that dysregulation of the IL-10/IL-12 balance play a crucial role in the impaired cellular immune responses seen in patients with SLE.

Defective immune regulatory mechanisms, such as the clearance of apoptotic cells and complexes, were important contributors to the development of SLE [59]. MRL/lpr mice are characterized by the presence of the lpr gene, which is associated with Fas (CD95) receptors on the surface of lymphocytes. The interaction of Fas and Fas ligand (Fas L) transduces an active signal for cellular apoptosis [90]. Defective Fas-mediated apoptosis in MRL/lpr mice results in massive lypmphoproliferation and the development of a severe lupus-like disease with immune glomerulonephrites [91]. Gld/gld mice, characterized by a mutation in the Fas L gene leading to a non-functional Fas L molecule, also develop lymphoproliferation, hypergammaglobulinemia and immunoglobulin deposits in the kidney [92]. Patients or mice with homozygous C1q deficiency develop autoantibodies and lupus-like syndrome apparently because of the inability to eliminate apoptotic cells effectively, which leads to an increase in the exposure of antigens to the immune system [93]. Mice with a targeted deletion of C1q show glomerulonephritis with deposits of immune complexes and apoptotic cells in the glomerulus.

In addition, environmental factors, such as chemicals and drugs, UV light, dietary factors, viruses and oestrogen are probably required to participate at the onset of the disease [94].

**Role of CE in the aetiopathogenesis of SLE**

Oestrogen (mainly 17β-oestradiol, oestradiol) and their metabolites seem to play an important role in SLE and autoimmune rheumatic disease [95]. A role for oestrogens in the pathogenesis of SLE has been suspected for many years [96]. SLE is 9 times more common in women than in men and 15-times more common during the childbearing years. Patients with Klinefelter’s syndrome (i.e. males with 47 chromosomes and an XXXY complement by karyotype analysis) are hyperoestrogenic and have an increased incidence of SLE [96]. Studies have revealed an abnormal pattern of oestrogen metabolism among SLE patients whereby the 16α-hydroxylation pathway is used as the major catabolic route [95, 98–100]. This pathway generates metabolic intermediates that function as reactive haptens capable of forming covalent adducts with epsilon amino groups of lysine residues via a Heyns-type rearrangement [101]. These conjugated products are thought to elicit the production of anti-oestrogen antibodies. Numerous case reports may be found in the literature demonstrating an association between the administration of certain synthetic oestrogens which are largely catabolized by the 16α-hydroxylation process.
route, e.g. ethyl-oestradiol and the onset of a drug-induced lupus-like syndrome [98, 102].

Metabolism of oestrone and oestradiol to hydroxylated end product is also a modifiable process. The ratio of high to low-potency oestrogen is 20-fold higher among patients with SLE than among healthy controls. CYP1A1 hydroxylate oestrogens into the biologic effectors of low potency (2-hydroxyoestrone). Greater CYP1A1 activity results in less available oestradiol for metabolism into high potency forms (16 α-hydroxyoestrone). CYP1A1 is an inducible enzyme [103]. Enzyme induction occurs in the presence of indole-3-carbinol, an organic compound found in cruciferous vegetables and omega-3-fatty acids. These two dietary factors have proved to be preventive in the development of ER-positive adenocarcinoma of breast, another condition with SLE-like ratios of high to low potency oestrogen [103, 104]. Indole-3-carbinol is currently under investigation for its disease-modifying potential of SLE. In addition, patients with SLE metabolize oestrogen in patterns that are distinct from those observed in unaffected controls. Cytochrome P450 isoenzymes CYP1B1 and CYP1A1 convert oestradiol to the biologic effectors 16 α-hydroxyoestrone and 2 α-hydroxyoestrone, respectively. The alterations in oestrogen metabolism have been reported in patients with SLE [98], suggesting that abnormal oestrogen metabolism is among the most biologically active serum oestrogens with a tumour growth-promoting effect. It activates B and T cells, induces transcription and promotes cell division. In contrast, the 2α-hydroxy product has little biologic effect [103]. Patients with SLE have increased activity of CYP1B1, with preferential hydroxylation of oestradiol to the more feminizing 16α-hydroxy metabolite [58, 105]. The altered conversion results in a 20-fold increase in the fraction of high- to low-potency oestrogen in patients with SLE when compared with healthy control [105].

16α-Hydroxyoestrone is a mitogenic and proliferative endogenous hormone that covalently binds to the oestrogen receptor leading to nuclear translocation [106]. Conversion products of oestrone and oestradiol are the 2-hydroxylated oestrogens such as 2-hydroxyoestrone and 2-hydroxyoestradiol. In contrast to 16-hydroxylated oestrogens, the 2-hydroxylated forms inhibit growth-promoting effect of oestradiol [107]. The altered conversion results in a 20-fold increase in the fraction of high- to low-potency oestrogen in patients with SLE when compared with healthy control [105].

Hydroxylated metabolites of oestrone and oestradiol are the 2-hydroxylated oestrogens such as 2-hydroxyoestrone and 2-hydroxyoestradiol. In contrast to 16-hydroxylated oestrogens, the 2-hydroxylated forms inhibit growth-promoting effect of oestradiol [107]. The study shows that urinary concentrations of the 2-hydroxylated oestrogens were 10 times lower in patients with RA and SLE than in healthy controls, whereas the ratio of 16-hydroxyoestrone/2-hydroxyoestrogens was 20-times higher in RA and SLE than in controls [95]. Inspite of that, Weidler et al. [94] showed that disease activity in SLE was negatively correlated with urinary concentration of 2-hydroxylated oestrogen. In a patient with RA and SLE, the magnitude of conversion to the mitogenic 16α-hydroxyoestrone is greatly up-regulated, which likely contributes to maintenance of the proliferative state in these diseases. Elevated serum concentrations of 16α-hydroxyoestrone have been described in patients with SLE [98], suggesting that abnormal patterns of oestriol metabolism may lead to increased oestrogenic activity. Similarly, there was a significantly higher concentration of 16-hydroxyoestrone/4-hydroxyoestradiol in the synovial fluids of patients with RA as compared with control fluids [108]. The alterations in oestrogen metabolism have been reported in both male and female patients [109, 110]. Peripheral oestrogen hydroxylation was found to be increased in both men and women with SLE and oestrogenic metabolites were reported to increase B-cell differentiation and activate T cells [111].

Oestradiol was found to increase IgG and IgM production by PBMCs from patients with SLE which led to elevated levels of polyclonal IgG, including IgG anti-double strand DNA, by enhancing B-cell activity via IL-10 [112]. To have a better insight into the possible role of CE in the aetiopathogenesis, we have demonstrated that CE-modified DNA was highly recognized by SLE IgG [22, 23], indicating the possible participation of modified DNA in SLE pathogenesis as it has been reported that CE-modified DNA bases cause DNA strand breakage and adduct formation in vivo and in vitro [113]. Hydroxylated metabolites of oestrogen have been detected in tissue, blood, bile and urine in humans [3]. The spontaneous production of anti-DNA autoantibodies in SLE might arise as a consequence of antigenic changes in DNA. Therefore, it could be possible that CE-modified bases of DNA might be one of the contributing factors towards the production of autoantibodies. In addition, oestrogen not only induces ROS production, but also modulates immune response and immunosuppressed disease [50]. It might be possible that oestrogen causes T- and B-cell differentiation and increases immunoglobulin production. These autoantibodies could be more strongly bound with modified DNA than native polymer. These studies show that hydroxylated oestrogen might have a role in the aetiopathogenesis of SLE and other autoimmune diseases.

Conclusion

It is well appreciated that oestrogen has profound influence on numerous tissues and in the development of various autoimmune diseases. It has become increasingly accepted that we must not only consider the parent oestrogens, oestradiol and oestrone, when we evaluate disease risk (autoimmune disease) but also the oestrogen metabolites (2- and 4-hydroxylated metabolites) should be taken in consideration. The CE metabolites, when oxidized to the electrophilic CE-Q, may react with DNA to form stable and depurinating adducts. The 4-CE that form predominantly depurinating adducts are carcinogenic, whereas the non-carcinogenic 2-CE exclusively form stable DNA adducts. Oxidation of CE also leads to overwhelming amount of ROS that generate extensive DNA damage, alter its immunogenicity, thus leading to the induction and elevated levels of SLE autoantibodies. Therefore, it could be possible that CE-modified bases of DNA might be one of the contributing factors towards the production of SLE autoantibodies.

The risk of developing SLE is higher among women compared with men. The role of oestrogen may provide a partial explanation for the aetiopathology of SLE but many questions remain. Females exhibit higher levels of serum IgG than males and mount a more vigorous humoral immune response [114]. PBMCs derived from patients with SLE, when treated with oestrogen undergo polyclonal activation, secrete anti-DNA IgG and display diminished apoptosis [115]. In addition, oestrogen not only induces ROS production but also modulates immune response and immune-mediated diseases. It might be possible that oestrogen causes T- and B-cell differentiation and increases IgG production. These autoantibodies could be strongly bound with DNA and serve as an immunochemical marker for the diagnosis of this disease.

The preliminary data presented in this review lead to the conclusion that CE (including other metabolites of oestrogen) might have a role in the pathogenesis of SLE. The results might also be replicated in the presence of 16-hydroxyoestrone as well as other hydroxylated metabolites to know the exact aetiopathology. SLE is a potential model for autoimmune disease in general and a model for complex diseases affected by numerous factors including hormonal interaction. Only by understanding the complexity of these interactions will we ultimately understand the aetiopathology of SLE.

**Rheumatology key messages**

- This review describes the possible role of CE in the aetiopathogenesis of SLE.
- This review describes the mechanism behind the generation of autoantibodies in SLE including the role of CE.
- This review is considered as the preliminary search of literature that may unfold various aspects of SLE.
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