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Research Article

Comparison of Estrogen and/or Progesterone on the Oxidative Status in Different Female Cancer Cells

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Abstract

Background: Oxidative stress has a key role in the development of most types of cancers. Among all the conditions which affect the antioxidant defenses, the effect of using oral contraception pills (OCP) or sex hormone therapy in women during menopause is still an unsolved issue.

Objectives: In the present study, the effect of estrogen (EST) alone, progesterone (PRO) alone and EST+PRO together on oxidative status in HeLa, MCF-7 and OVCAR-3 cell lines was investigated.

Methods: HeLa, MCF- 7 and OVCAR-3 cell lines were treated with 1μM of EST, PRO and EST+PRO confirmed by MTT and used for 24 hour treatment. Then, catalase (CAT), glutathione (GSH), glutathione peroxidase (GPX), glutathione reductase (GR) activities and Malondialdehyde (MDA) levels were assayed in the cell lysate.

Results: The results of the present study indicated that EST, PRO, and their combination were strongly able to increase the antioxidant enzymes activity compared with controls and significantly decreased the MDA levels in all three cell lines.

Conclusion: The data suggest that female sex hormones and OCPs might exert antioxidant effects in different cancerous organs.

Keywords: Oxidative Stress, Estrogen, Progesterone, Antioxidant Enzymes, Female Cancers

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1. Background

Over the past two decades, a large number of studies have shown how constitutive oxidative stress which is defined as an imbalance between antioxidant components and reactive oxygen species [1–4] can lead to chronic inflammation, possible cancer formation and ultimately cancer metastasis [2, 5]. In fact, excessive free radicals actively interfere in cell proliferation and cause serious damage to the membrane, mitochondria along with macromolecules [6, 7]. This type of damages usually acts as a signal to trigger an inflammatory response and activate a variety of transcription factors that are involved in the over-expression of different genes including growth factors and cell cycle genes [7, 8] which can lead to cancer. The oxidative stress pivotal role in breast tumor, human cervix carcinoma cancer, and ovary carcinoma cancer has been previously reported [9].

Among the conditions known to somehow affect the oxidative stress, the use of OCP in women has been proposed. OCP or Combined oral contraceptive pill (COC) and progesterone only pill (POP) are hormonal contraceptive methods which are effective in preventing pregnancy with a convenient, safe, and reversible method of contraception [10]. These pills are used primarily to inhibit pregnancy through disrupting endogenous endocrine function [11]; however, owing to the obscure effect of the pills on the antioxidant complex defense, the use of this method is still controversial and the fear of cancer and cardiovascular disease overshadows its usage [9, 12]. The use of COCPs has been reported to reduce the activity of antioxidant enzymes in the serum [13, 14], but there are some studies that have presented completely the opposite results [14, 15].

COCPs contain a synthesized form of estrogen, and progesterone [14]. Studies on the effects of estrogen and progesterone on the oxidative stress, as two effective factors in these pills, have shown that antioxidant enzymes are one of the major target genes for progesterone and estrogen in oxidative stress [16, 17].

Therefore, with the existing contradictions, further studies on oxidative stress in different human tissue cells and under the influence of contraceptive pills are strongly recommended. Therefore, this study aimed to investigate the effect of EST alone, PRO alone and EST +PRO together on the activity of antioxidant enzymatic markers and MDA levels, as a marker of lipid peroxidation, in three major cell lines including the cervix, breast, and ovarian cancer cell lines.

2. Materials and Methods

2.1. Materials

Dimethyl sulfoxide (DMSO), oxidized glutathione (GSSG), tert-butyl hydroperoxide (t-BuOOH), glutathione reductase (GR), bovine serum albumin (BSA), nicotine-amid-adenine-dinucleotide phosphate (NADPH), triton X-100, TEP (1, 1', 3, 3′-tertaethoxy propane) were purchased from Sigma Chemical Co (Poole, Dorset, UK). RPMI, FBS (fetal bovine serum), DMEM, streptomycin and penicillin were purchased from Gibco-BRL (Paisley, UK). EST and PRO were obtained from Abouraihan Darou (Tehran, Iran).

2.2. Cell Culture and Treatments

Human cervix carcinoma cancer cell line (HeLa), ovary carcinoma cancer cell line (OVCAR-3) and breast carcinoma cancer cell line (MCF-7) were provided by Pasteur Institute of Iran. HeLa and MCF-7 cells were grown in RPMI-1640 media with 10% fetal calf serum, (100 μg/ml + 100 U/ml streptomycin and penicillin at 37°C in 5% CO_2). For OVCAR-3 cell line media supplement was DMEM-HG. The cells were seeded at a density of 800,000 cells in a 25 cm_2 flask and all experiments were done using cells in passage numbers 2–5 at 70% confluency. Then, the cells were treated with 0.1% ethanol as vehicle, EST (1μM) and/or PRO (1μM) in comparison with a control group.

2.3. MTT Assay

All cell viability assay was performed using 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) as described previously [18], with some modifications. Briefly, HeLa, MCF-7 and OVCAR-3 cells (10,000cells/well in 96-well plate) were first incubated with applied treatments for 24 h at 37ºC. The cells were incubated in serum-free medium and MTT (0.5 mg/mL, 10 μl) was added. After 3.5 hours of incubation, DMSO (100 μl) was added to dissolve the formazan crystals and the absorbance was determined at 570/650 nm with ELISA reader.

The number of metabolically competent cells was determined as the ratio of absorbance of the treated cells to untreated cells which served as the control (expressed as a percentage). The experiment was repeated five times.

2.4. Glutathione Peroxidase Activity Assay

GPx activity in clear supernatant cell lysate was assayed by the procedures of Fecondo and Augustey [19] with minor modifications [20]. The enzyme activity was expressed as μm of NADPH oxidized/min/mg cell protein, using a molar extinction coefficient of 6.22×106 M-1cm-1 for NADPH. One unit of GPx was defined as mU/mg cell protein.

2.5. Glutathione Reductase Activity Assay

The Racker method was used for GR activity assay, with minor modifications [21]. The decrease in absorbance at 340 nm, which reflects the oxidation of NADPH during reduction of GSSG by GR present in the sample, was monitored for 3 min with a spectrophotometer. Using a molar extinction coefficient of 6.22×106 M-1cm-1 for NADPH, one unit of GR was defined as mU/mg cell protein.

2.6. Determination of GSH

The assay of GSH with DTNB was performed followed by a standard Ellman's method with minor modifications [22, 23]. GSH level was analyzed in the clear supernatant of the cell lysate. Potassium phosphate buffer (0.2 M, pH 7.6) and DTNB (0.001 M) were added to 0.2 ml of cell lysate supernatant respectively. An absorbance of the products was observed at 412 nm after 5 min.

2.7. Catalase Activity Assay

The method of Aebi [24] was used for CAT activity determination, using an ultraviolet spectrophotometer (UV- 160A, Shimadzu, Japan). The H_2O_2 decomposition rate was monitored at 240 nm. Molar absorptivity of 43.6 L.mol-1.Cm-1 was used to calculate the enzyme activity. 1 µm of H_2O_2 decomposition/min was equal to One unit.

2.8. Estimation of MDA and Protein

MDA, as a marker of oxidative stress, was measured according to the Placer method. Briefly, the samples were precipitated with a mixture of TCA and TBA and boiled. The supernatant was collected; then, we measured absorbance at 535 nm [25]. The results are expressed as units of enzyme activities per mg of protein. The protein concentration was measured by Bradford method [26], using BSA (1mg/1ml) as standard.

2.9. Statistical Analysis

The effect of different treatments was assessed on various parameters, using repeated measures statistical design. All data were expressed as mean ± SEM and analyzed using Kruskal-Wallis and Mann-Whitney tests for group comparison. $P<0.05$ was considered as statistically significant. SPSS 16 software was used for data analysis.

3. Results

3.1. Effect of Treatments on Cell Survival in HeLa, MCF-7 and OVCAR-3 Cell Lines

The viability of cultured HeLa, MCF-7 and OVCAR-3 cells exposed to various concentrations of EST and PRO was evaluated through the MTT assay. The result showed that in all tested concentrations, cell viability was more than 80%, so based on similar studies we used the 1µM dose of EST and PRO.

3.2. Effect of Treatment on the CAT Activity

As demonstrated by Figure **1**, treatment with EST, PRO and EST+PRO significantly increased the CAT activity compared with the controls (P<0.05). Also, it was indicated that the elevation of CAT activity in the cells treated with EST was dramatically higher than treatment with PRO. These results can be generalized to all three cell lines, as shown in Figure **1**.A, B, and C. However, no significant difference was observed between the EST and EST+PRO treated groups in MCF-7 and OVCAR-3.

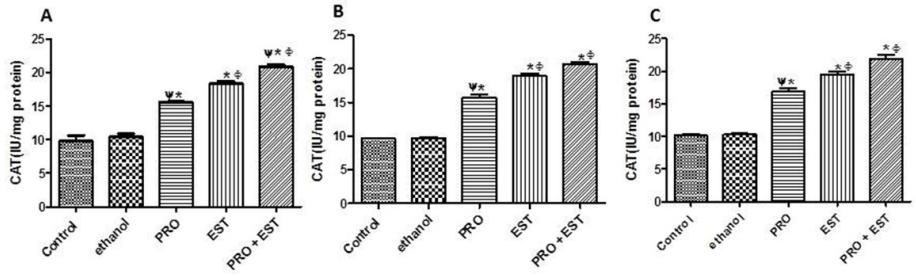

Figure 1: Effect of treatment on the activity of **CAT** in **A.HeLa**, **B.MCF-7**, and **C.OVCAR-3** cells. Data are presented as mean ± SEM. *P < 0.05 for significant change compare with the control group, Φ indicates significant differences in compare with the PRO group, and Ψ shows significant changes in compare with the EST group.

3.3. Effect of Treatment on GPx Activity in Three Cell Lines

Figures **2**.A, B, and C display the summary statistics for analysis of GPx activity in three cell lines and three different conditions. The intracellular GPx activity was increased by 63.6% and 85.5% for HeLa cells treated with EST and EST+PRO, respectively (*P*<0.05). PRO had no significant effect on this cell lines compared with the controls (Figure **2**A). However, all 3 treatments significantly induced GPx activity in MCF-7 (Figure **2**B) and OVCAR-3 cell lines (Figure **2**C), compared with the controls.

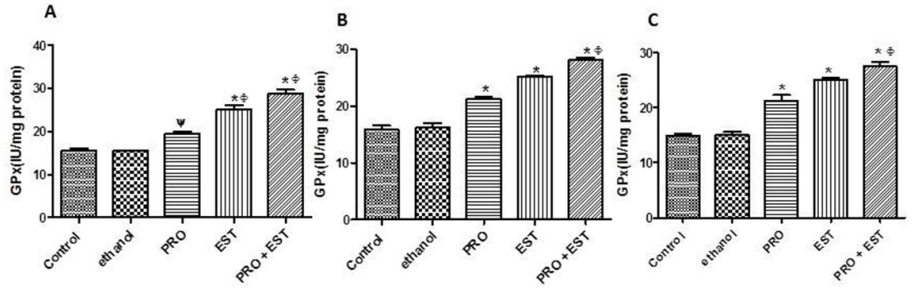

Figure 2: Effect of three different treatments on the activity of **GPX** in **A. HeLa**, **B.MCF-7**, and **C.OVCAR-3** cell lines. * For significant change compare with the control group (P < 0.05), Φ indicates significant differences in compare with the PRO group, and Ψ shows significant changes in compare with the EST group. Data are presented as mean ± SEM.

3.4. GR Activity Assessment after Three Different Treatments

The treatment of cells with the 1μM dose of EST and PRO and EST+PRO drastically increased the GR activity in MCF-7 cell line by 34.0%, 57.1%, and 70.1%, respectively as compared with the controls (Figure **3**B). It is noteworthy that in all three cell lines, increased activity of the GR after treatment with EST was significantly higher than PRO condition.

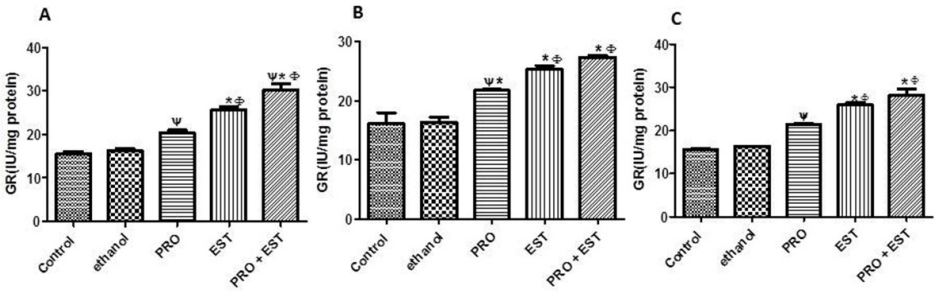

Figure 3: Effect of EST, PRO, and EST+PRO treatments on the activity of **GR** in the **A. HeLa**, **B.MCF-7**and **C.OVCAR-3** cell lines. Significant changes compare with the control group showed by * ($P < 0.05$), Φ indicates significant differences in compare with the PRO group, and Ψ shows significant changes in compare with the EST group. Data are presented as mean ± SEM.

3.5. Effect of Treatment on GSH Levels

The GSH level in HeLa cell lysate in all 3 conditions was remarkably increased compared with the control group (Figure **4**A). Despite the increased level of GSH after treatment with PRO in MCF-7 and OVCAR-3 cell lines, it was not significant as compared with the controls.

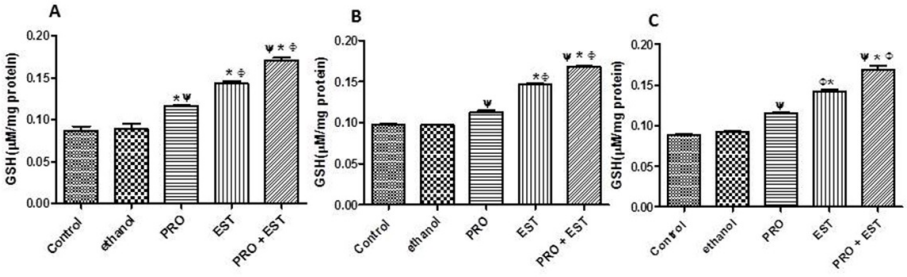

Figure 4: Effect of three different treatments on the **GSH** levels in **A. HeLa**, **B.MCF-7**, and **C.OVCAR-3** cell lines. * For significant different compare with the control group ($P < 0.05$), Φ show significant differences in compare with the PRO group, and Ψ indicates significant changes in compare with the EST group. Data are presented as mean ± SEM.

3.6. MDA Levels as a Marker of Lipid Peroxidation

It was revealed that the MDA levels significantly decreased in all cell lines and after treatment with PRO, EST and their combination compared with the control group (no treatment). Furthermore, what is striking

about the figures is that EST was more potent to decrease the MDA levels compared to PRO and this applies to all the cell lines (Figure **5**A, B, and C).

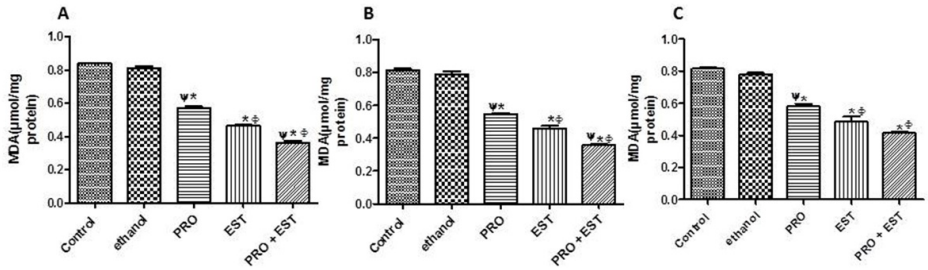

Figure 5: Effect of three different treatments on the **MDA** levels in **A. HeLa**, **B.MCF-7**, and **C.OVCAR-3** cell lines. * For significant change compare with the control group ($P < 0.05$), Φ indicates significant differences in compare with the PRO group, and Ψ shows significant changes in compare with the EST group. Data are presented as mean $\pm$ SEM.

4. Discussion

Among various contraceptive methods, COCPs are the most common, convenient and reversible ways of contraception women have ever used [27].

Our data showed that both EST and PRO can be a powerful enhancer of antioxidant enzymes activity; however, there is no evidence for any difference in their effects depending on the types of tissues, and we've seen almost the same changes in all three cell lines. Noteworthy, based on our results, EST was found to be much more potent than PRO. Besides, the combination of PRO with EST, which is also found on COCPs, has been shown to have the remarkable vigor for increasing the antioxidant defense power. Our data broadly support the previous findings [28]. Chang et al. (2015) showed that the elevation of EST concentration led to a significant increase in the GSH level and this also applies to other antioxidant factors that we examined in this study [29–31]. In Tang et al.'s study, the use of 4-hydroxy estrone during lipid peroxidation processes resulted in lower MDA level which is a reliable marker of lipid peroxidation [32, 33]

ROS are generated in aerobic processes in moderated amounts and are needed for signaling pathways in the many cells [34]. however, its overproduction can lead to oxidative stress, severe cellular damages, and end up with cell dysfunction or death [35]. Hence, the antioxidant defense system in the cells is responsible for suppressing the dangerous effects of ROS and reducing the risk of developing cancer indirectly.

Therefore, improving the complex antioxidant defense by female sex hormones prevents the oxidative stress and intense cell damages, and may reduce the chance of cancer in women. Although the exact mechanism by which sex hormones can affect the activity of antioxidant enzymes has not been identified yet, it has been suggested that antioxidant genes are partly under the control of sex hormones [36].

Previous studies have revealed that GPx, CAT, and GR might be the target genes for PRO which protect the cells from oxidative stress by upregulating this genes [10, 37]. Also, EST has been shown

to activate MAPK and NF-κB, driving the expression of GPX antioxidant enzyme [14, 38]. Additionally, estrogen replacement therapy in menopause women, which is often done to reduce the complications of menopause, led to modified antioxidant enzyme levels by acting as regulators of key antioxidant gene expression [1, 39, 40]. However, this needs to be further studied.

5. Conclusion

Based on our findings, ESR and PRO, which are also used combined together in the COCPs, play a protective role against the oxidative stress in different female tissues, especially in cancerous conditions. The results suggested that administration of COCPs, as an easy way of contraception, surprisingly not only does not increases the risk of cancer, but also is able to reduce the complications of oxidative stress in women by strengthening the antioxidant defense.

Implications

Administration of COCPs as an easy way of contraception, surprisingly able to reduce the complications of cancer in different female organs.

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Disclosure Statement

The authors don't have any conflict of interest.

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