EFFECT OF ORAL-ESTROGEN CONJUGATE ON B-LYMPHOCYTE PROLIFERATION IN THE PEYER’S PATCHES OF ADULT FEMALE BALB/C MICE ILEUM

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ABSTRACT

This study was aimed to analyse the effect of oral-estrogen conjugate on B-lymphocytes proliferation that is part of humoral immune system. Modulation of B-lymphocyte number and the germinal centre diameter in peyer’s patches of adult female BALB/C mice ileum (age 2-3 months) were analysed by diastase PAS staining. A total of 40 mice were divided equally into 4 groups (Cerel-Suhl et al. 1999); the 3-weeks treated group and its control group (Prabowo 1997), the 6-weeks treated group and its control group. Each treated group was given oral-estrogen conjugate emulsion (0.001625 mg/20 gram of bodyweight/days). Data were analysed using unpaired t-test with Levene test and Pearson correlation test, p<0.05 was considered significant. There was a significant difference on the germinal centre diameter of ileum peyer’s patches between the 3-weeks treated group and its control group (p<0.05), whilst both B-lymphocyte number and germinal centre diameter of ileum peyer’s patches in the 6-weeks treated were significantly different compared to those of its control group (p<0.05). In conclusion, oral-estrogen conjugate may modulate component of the immune system. Further studies are needed to seek the duration and clinical effect of these modulations on the overall immune system.

Keywords: oral contraceptive-pills, cellular and humoral immune response

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INTRODUCTION

Estrogen is widely used in various drugs component, as in contraceptive hormone preparations where estrogen available for both oral and injection use. Estrogen that is found in contraceptive pills, usually combined with progesterone. Nowadays, oral-contraceptive pills are highly preferred due to their effectiveness and practical using, whilst have reversible side effect. Non-contraceptive effects of estrogen are also considered beneficial amongst the users; it can be used as oral-therapy of dysmenorrhea, metrorragia, pre-menstrual syndrome (PMS), hirsutism, ovarian and endometrial cancer, ovarian cyst, ectopic pregnancy, acne, and endometriosis (Cerel-Suhl et al. 1999). Other use of estrogen is as a hormone replacement therapy (HRT), especially for menopausal woman with various complaints caused by decreased endogenous estrogen levels. Estrogen replacement therapy (ERT) has been used as one way of preventing osteoporosis especially in post-menopausal women since 1960’s (Prabowo 1997). The advantage and disadvantage of using ERT have been widely studied.

Grimaldi et al. demonstrated that mature and immature B-lymphocytes express both alpha and beta estrogen receptors (Grimaldi et al. 2002). The alpha receptors are more predominantly expressed by the transitional and
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MATERIALS AND METHODS

A total of 40 BALB/C female mice age 2-3 months were divided equally into 4 groups; the 3-weeks treated group, the 6-weeks treated group and control groups for each treated group. Each treated group was given oral-estrogen conjugate emulsion (Premarin, dosage of 0.001625 mg/20 gram of bodyweight/days, once a day-morning dose) for three and six weeks respectively, whereas the control groups were given placebo (emulsion of gum Arabica without oral-estrogen conjugate). At the end of the treated, mice were sacrificed by lethal cervical fracture; mice were dissected and the ileum was removed. The organ was processed by paraffin method for histology preparation. Diastase periodic acid Schiff (diastase PAS) staining was applied on the processed-ileum tissue slides to identify mature B-lymphocytes that cytoplasm was stained magenta; indicating a high-glucose content of these cells (40x objective lens). Longitudinally cut ileum tissue section slides of 8 micrometer from each mouse were brought to distilled water prior to periodic acid treatment for 5 minutes. After washing well in distilled water, staining with Schiff's reagent for 10 minutes was applied and then washed under running tap water for 3-5 minutes. Lastly, sections were dehydrated, cleared and mounted. The B-lymphocytes count was done using ToolImage software. The diameter of the germinal centre in the ileum peyer’s patches was measured one by one using the micrometer with 10x objective magnification. All histological analysis were done using the light microscope (Olympus, Japan). Images obtained were processed by Adobe Photoshop CS3 for minor adjustment. Statistical analysis were done using unpaired t-test with Levene test and Pearson correlation test (SPSS 9.0). p<0.05 was considered significant.

RESULTS

Figure 1 showed the measurement of germinal centre diameter in ileum peyer’s patch using 10x objective magnification. We evaluated all of the ileum peyer’s patch that was found in each mouse, where 1 mouse generally has 1 observed peyer’s patch in its ileum. The germinal centre appears as pale staining area in centre of primary follicle using diastase PAS staining (area crossed by the micrometer in Fig. 1), where the longest diameter of each germinal centre was measured and statistically compared. Figure 2 showed the mature B-lymphocyte which cytoplasm was stained magenta using diastase PAS, indicating high-glucose content of this cell.

Table 1 showed that the average of germinal centre diameter and number of B-lymphocytes in each treated group is larger than their respective control groups, both in three weeks groups and six weeks groups. The germinal centre diameter is significantly increased in both treated groups compare to their respective control groups (p<0.05). On the other hand, no significant differences between the three weeks treated group and six weeks treated group.
Table 1. Germinal centre diameter (micrometer) and number of mature B-lymphocytes in the treated and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Three weeks groups</th>
<th></th>
<th>Six weeks groups</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Treated group</td>
<td>Control group</td>
<td>Treated group</td>
</tr>
<tr>
<td>Germinal centre diameter</td>
<td>372.00±57.27\textsuperscript{a}</td>
<td>487.00±100.12</td>
<td>308.57±60.07\textsuperscript{b}</td>
<td>510.71±115.34</td>
</tr>
<tr>
<td>Number of mature B-lymphocytes</td>
<td>4.80±3.11</td>
<td>6.00±1.22</td>
<td>2.86±1.57\textsuperscript{c}</td>
<td>8.71±2.81</td>
</tr>
</tbody>
</table>

Note: the superscript show where the significant differences between each treated and its control group (p<0.05).

DISCUSSION

Lymphocytes, the cells that are responsible for the specificity and memory in adaptive immune response, produced in the primary lymphoid organs and function in secondary lymphoid organs/tissues (Grimaldi et al. 2001, Lydyard et al. 2000, Cormack et al. 1992, Abbas et al. 1991). There are three types of lymphocytes – NK cells, T cells and B cells, although only T and B cells have true antigen specificity and memory (Grimaldi et al. 2001, Lydyard et al. 2000, Cormack et al. 1992, Abbas et al. 1991). B cells are produced in bone marrow and migrate to secondary lymphoid organs where they respond to foreign antigens (Grimaldi et al. 2001). Proliferation and differentiation of B-lymphocytes can be observed in the gut-associated lymphoid tissue (GALT), especially in germinal centre of ileum peyer’s patches (Lydyard et al. 2000, Cormack et al. 1992). Activated B-lymphocytes will proliferate in the germinal centre that may result in increased germinal centre size and diameter. In addition, B-lymphocytes differentiate into plasma cells that produce circulating immunoglobulin A and memory cells (Cormack et al. 1992, Abbas et al. 1991).

Various stage of B-lymphocytes can express the estrogen receptor, including mature, immature and autoreactive B-lymphocytes (Grimaldi et al. 2002, Thurmond et al. 2000). Estrogen can influence B-lymphocytes through increasing the expression of genes that are responsible for B-lymphocytes survival (Grimaldi et al. 2002, Thurmond et al. 2000). Estrogen also has role in development of hematopoietic progenitors and B lymphocytes maturation showed in the male mouse (Cormack et al. 1992). Estrogen alters B-cell development to decrease lymphopoiesis and increases the frequency of marginal zone cells, including increases the maturation and activation of autoreactive marginal zone B cells in model estrogen-induced lupus mice (Grimaldi et al. 2001, Abbas et al. 1991). The engagement of Estrogen receptors to Estrogen can alter B-cell maturation, although engagement of Estrogen receptor alpha only that likely has a potential to trigger the autoimmunity conditions.
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In three weeks treated group where the number of mature B lymphocytes was not significantly different to the control group, the oral-conjugated Estrogen given may not be strongly enough to cause survival modulation undergone onto mature B-lymphocytes in the germinal centre. Whilst in the six weeks treated group, the 6 weeks exposure of estrogen may already influence the survival rate of mature B-lymphocytes. The autoreactive B-lymphocytes that also express estrogen receptors may also be engaged to the Estrogen given, this may lead to increase the potential for autoimmunity occurs. Normally, these cells will be deleted in the process of B lymphocytes maturation (Grimaldi et al. 2002, Hill et al. 2011, Lydyard et al. 2000, Cormack et al. 1992).

In three weeks treated group, the germinal centre diameter increase significantly, which may indicate increased proliferation of B-lymphocytes when compared to the control group. Interestingly, the number of mature B-lymphocytes did not increase significantly in the three weeks treated group compared to its control group. This may mean that the increased germinal centre diameter is likely due to the hypertrophy of mature B-lymphocytes and/or proliferation occurred upon other than the mature B-lymphocytes, which in this case can not be detected by diastase PAS. In the six weeks treated group, both germinal centre diameter and number of mature B-lymphocytes were increased significantly when compared to the control group. We suggest that the increased germinal centre diameter is likely due to the mature B-lymphocytes proliferation, whilst the later may be caused by normal maturation process, effect of oral-estrogen conjugate and/or both.

The significant larger germinal centre diameter between each treated group and their respective control groups seen in the current study was probably due to upregulation of the apoptosis B-lymphocytes regulatory genes (CD 22, SHP1, Bcl-2, VCAM1) by the Estrogen (Grimaldi et al. 2002, Medina et al. 2000). These changes lead to increasing the survival rate of high affinity anti-DNA against B-cell receptor (BCR)-mediated apoptosis on immature, transitional and autoreactive B-lymphocytes (Grimaldi et al. 2002, Medina et al. 2000). This condition causes the various stages of activated B lymphocytes do not undergo apoptosis immediately (Grimaldi et al. 2002, Medina et al. 2000).

CONCLUSION

Oral-Estrogen conjugate can alter component of immune system shown in the germinal centre of ileum peyer’s patch of adult female BALB/C mice. The changes are shown as modulation of the germinal centre diameter and number of mature B-lymphocytes in specific period treatment.

REFERENCES