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Comparing the Effects of MePB, BuPB, BenzPB on Receptor-α and -β and the Progesterone Receptor in Human Breast Cells and in Mice

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Abstract

Ever since paraben was first discovered to possess estrogenic properties in 1998, various studies have shown that they can inhibit 17β-HSD1, which catalyzes the final step of estrogen biosynthesis. This study compares the difference in the performance of parabens on estrogen and progesterone receptors in both in vitro in human MCF-7, MCF-10a and through topical treatment in vivo in mice using western blot. The experiment uses two human breast cell line and in vivo study of mice with topical application of different concentrations of paraben. The level of ER and PGR protein expression is tested using western blot. The study aims to compare the estrogenic activity of parabens in human and mice to show that parabens are one of the potential causes of cancer initiation and progression. The study provides data for the different effect of parabens and evidence for future studies relating to cosmetic products.

Keywords

Parabens; Breast Cancer; ER-α; ER-β; Protein Expression in Human and Mice.

1. Introduction

The alkyl esters of p-hydroxybenzoic acid, also known as parabens, are widely used in industries like food, cosmetics and pharmaceutical products as preservatives because of their antimicrobial property and low toxicity. Over the past decades parabens are considered to be relatively safe compounds and to have a low bioaccumulation potential. [1] However, concerns has been raised towards its safety since the first measurement of intact esters of p-hydroxybenzoic acid (parabens) in human breast cancer tissues, which suggests that topical application of cosmetic products could be a possible factor contributing to the initiation and development of breast cancer. [2] After that different researchers have detected the existence of parabens in human tissue fluids including urine, blood, milk and human breast tumours in various populations. [1]

Breast cancer is the second most common cancer and the commonest cause of cancer death in women worldwide. The imbalance of sex hormone is likely to induce breast cancer [3], so the role of estrogen (17β-estradiol) and progesterone (P4) receptors is crucial in the cancer initiating process. The primary sex hormone estrogen, which controls mammary gland development, has two classes of receptors: nuclear estrogen receptors ER-α (ESR-1) and ER-β (ESR-2). [4] Progesterone functions using progesterone receptor (PGR), and it affects not only normal breast development but also the carcinogenesis of breast epithelium and progression of breast cancer. [5]

Ever since paraben was first discovered to possess estrogenic properties in 1998, various studies have shown that they inhibit 17β-HSD1, which catalyses the final step of estrogen biosynthesis. [6] Although in animal studies parabens are shown to be actively absorbed, metabolized and excreted as p-hydroxybenzoic acid, some studies have also shown that due to their high oil/water partition coefficient, there are chances for accumulation in fatty components if parabens enter the human body

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intact, and if so it would significantly increase expression of ESR and PGR at mRNA and protein levels, except for butylparaben.[1][7] According to ECHA paraben is currently under assessment as Endocrine Disrupting.[8] Therefore the purpose of this study is to compare the difference in the performance of selected parabens: MePB (Methylparaben), BuPB (Butylparaben), and BenzPB (Benzene paraben) (Fig.1) on estrogen and progesterone receptors in both in vitro in human mcf7, mcf10 and through topical treatment in vivo in mice using western blots to provide a better understanding of the possible role of parabens as estrogen disruptors and provide information for future regulation of cosmetic and food industries.

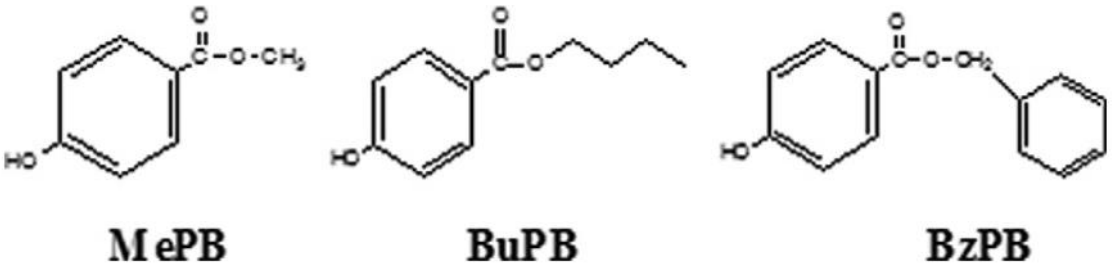

Fig 1. Chemical structures of MePB, BuPB and BzPB.

2. Materials and Methods

2.1 Chemicals and reagents

The parabens will be dissolved in glycerine due to the relatively higher solubility and to imitate their environment in cosmetic products, kept at 25 C°. The final concentration of the reagents will be methyl paraben (5, 10, 20 nM), butyl paraben (5, 10, 20 nM), benzene paraben (5, 10, 20 nM) and insulin (5nM) as positive control with shown ability to increase ER protein in MCF-7 cell line. [9]

2.1.1 The in vitro study

2 types of human cell lines will be used: MCF-7 cancerous breast tumour cells and MCF-10A noncancerous breast epithelial cell. The cells were cultured in RPMI medium with 1% (v/v) L-glutamine, 10% (v/v) fetal bovine serum, 1% (v/v) penicillin (10,000 IU/mL), and 1% (v/v) streptomycin (10,000 UG/mL) in a 5% CO2 atmosphere at 37 °C. [2] Positive is cultured in prepared insulin solution and DMSO was used as a negative control.

2.1.2 The in vivo study

110 mouse will be divided into 11 groups of 10. The negative control group is treated with topical application of glycerine at feet and their back daily. The positive control group injected with insulin and the rest applied with MePB, BuPB, BzPB solutions at different concentrations respectively. Collect breast cells from them at day 3, 6, 9 for further analysis. The mice were housed under specific pathogen–free conditions. The animal experiment will strictly follow the AAAALAC guidelines. Animals will be euthanized immediately if they display excessive discomfort.

2.2 Western blot

Whole cell extracts were washed with chilled phosphate-buffered saline (PBS), scraped, collected in PBS, and centrifuged at 1000g for 5min at 4◦C, and cell pellets were resuspended in 200µlof50mMTris–HCl, 20mM EDTA, 5% sodium dodecyl sulphate (SDS), 1mM phenylmethylsulphonyl fluoride (PMSF), 5mM β-glycerophosphate, and 1mM aprotinin. ER-specific mouse monoclonal antibodies1D5 (DAKO, Canada) and a horse-radish peroxidase (HRP)-conjugated goat anti-mouse antibody (Hyclone Laboratories, Logan, UT, USA) were used as primary and secondary antibodies. Western blot experiments were performed at least in triplicate on independent cell transfections. [10]

β-glycerophosphate, and 1mM aprotinin.

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2.3 Statistical analysis

The statistical significance of all numerical data collected from western blot will be evaluated using student's t test ($p<0.05$).

3. Possible results

3.1 Result 1: Increasing concentration of all parabens increased the protein expression of ER and PGR in both human cells and mice cells

3.1.1 The in vitro study of human cells

As concentration of MePB, BuPB, BzPB solution increases, more ER and PGR proteins are detected in the treated MCF-7 and MCF-10A cell culture in the western blot analysis. The number of proteins were all greater than the DMSO cell culture, which expresses the normal amount. The cell line treated with insulin solution also has increased ER and PGR protein expression, indicating normal function of western blot analysis.

3.1.2 The in vivo study in mice

From cells taken from MePB, BuPB, BzPB treated mice groups, expression of ER and PGR protein increases as concentration of solutions increases according to western blot analysis. The negative control group provided with glycerine showed the least protein expression of ER and PGR, which is the normal level. The positive control group injected with insulin showed higher level of ER and PGR protein expression than the negative control group.

3.2 Result 2: Increasing concentration of all parabens increased protein expression of ER and PGR in only human cells but not mice cells.

3.2.1 The in vitro study of human cells

The results are the same as 3.1.1

3.2.2 The in vivo study in mice

From cells taken from MePB, BuPB, BzPB treated mice groups, expression of ER and PGR protein did not change significantly as concentration of solutions increases according to western blot analysis. The negative control group provided with glycerine showed the normal level of protein expression of ER and PGR and has around the same amount of protein expression as the parabens treated groups. The positive control group injected with insulin showed higher level of ER and PGR protein expression than the negative control group.

3.3 Result 3: Increasing concentration of all parabens increased protein expression of ER and PGR in only mice cells but not human cells.

3.3.1 The in vitro study of human cells

As concentration of MePB, BuPB, BzPB solution increases, no significantly more ER and PGR proteins are detected in the treated MCF-7 and MCF-10A cell culture in the western blot analysis. The number of proteins were at relatively the same level as the DMSO cell culture, which expresses the normal amount. The cell line treated with insulin solution also has increased ER and PGR protein expression, indicating normal function of western blot analysis.

3.3.2 The in vivo study in mice

The results are the same as 3.1.2

3.4 Result 4: Increasing concentration of some parabens increased protein expression of ER and PGR in human cells but not mice cells

3.4.1 The in vitro study of human cells

The MCF-7 and MCF-10 cell line treated with MePB showed increase in ER and PGR protein expression according to western blot analysis. However, there is no significant difference in the amount of proteins between the cell lines treated with BuPB and BzPB solution compared to DMSO

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control group, which indicated the normal level. The positive control group treated with insulin showed increase in protein expression as expected.

3.4.2 The in vivo study in mice

The results are the same as 3.2.2

3.5 Result 5: Increasing concentration of some parabens increased the protein expression of ER and PGR in both human cells and mice cells

3.5.1 The in vitro study of human cells

The results are the same as 3.4.1

3.5.2 The in vivo study in mice

The mice group treated with MePB solutions had increased ER and PGR protein expression in their cells as concentration increase according to western blot analysis. The results of the groups treated with BuPB and BzPB and pure glycerine were pretty close, with the negative control group indicating the normal level. Positive group injected with insulin had increased ER and PGR protein expression as expected.

3.6 Result 6: Increasing concentration of all parabens did not increase protein expression in neither human cells nor mice cells, with positive control insulin increase in both sample.

3.6.1 The in vitro study of human cells

None of the MCF-7 or MCF-10A cell line treated with MePB, BuPB or BzPB showed significantly different amount of ER and PGR protein expression compared to the negative control group of DMSO cell line, regardless of changes in concentration. So none of the paraben solution had significant effect. The positive control group treated with insulin solution had increased level of protein expression as expected according to western blot analysis.

3.6.2 The in vivo study in mice

None of the cells from mice groups treated with parabens of different concentrations had increased ER or PGR protein expression in the western blot analysis. The results were very close to the negative control group treated with topical application of glycerine. The positive control group injected with prepared insulin solution showed increased level of protein expression.

4. Discussion

Previous studies have showed that parabens, although not yet classified as carcinogen, possesses estrogenic properties and will increase ER and PGR protein expression in human cancerous breast tumor MCF-7 cell line. To confirm whether parabens have the same effect on mice and human, this study compares the difference in the actions of three parabens, MePB, BuPB and BzPB in human cells *in vitro* and mice *in vivo*, using western blot analysis to detect any possible changes in protein expression of the estrogen and progesterone receptors.

There are mainly six possible results according to the designed experiment. The possible difference in actions of different parabens, whether the parabens have the same effect in human and in mice and whether paraben itself has significant influence are concluded and discussed below.

Possible result 1 fully supports the hypothesis of the study, it is also most consistent with previous researches. It shows that as more parabens accumulate in the body, they cannot be hydrolyzed completely and will inhibit estrogen synthesis. The lack of estrogen leads to increased ER protein expression, and cell proliferation is stimulated to take place more easily. [10] The result shows that parabens have the potential to initiate cancer or accelerate cancer progression in breast at a certain critical concentration.

The situation of possible result 2 and 4 might be due to the slightly different metabolisms in mice and in human. Studies have shown the mice have a much higher metabolism rate than human beings [11], so they might be able to decompose and hydrolyze the parabens much faster. Less parabens

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accumulating in their body leads to a less significant result. In this case future studies should refer less to animal studies and focus more on human cell lines to provide more specific results concerning human beings.

The possible reason leading to result 3 may be the different method delivering positive control insulin compared to topical application of parabens on mice. Direct injection into blood provides a much faster pathway for the chemicals to reach the body, providing much more significant results. In future studies a better positive control should be selected to avoid such results.

Result 4 and 5 shows that different parabens have different penetration and accumulation abilities. Smaller molecule of methyl paraben easily inhibits estrogenic pathway and provides significant data, but larger molecules like BuPB and BzPB failed to provide such data, due to their sizes and especially BzPB with a benzene ring. The possible failure of result 6 may be avoided if higher concentrations of different parabens are used. The current range of concentration is very limited and may be too low to show any significant difference in results.

5. Conclusion

The study compares the estrogenic activity of parabens in human and mice to show that parabens are one of the potential causes of cancer initiation and progression from the ER and PGR protein expression aspect using western blot analysis. The different possible results showed that a better delivering method of the chemicals are needed in future studies, and researches should concern various kinds of parabens when studying their actions due to their different structures. The study provides data for the different effect of parabens and evidence for future studies relating to cosmetic products, and casts doubt on the accuracy of animal experiments with their higher rate of metabolism. More investigation should be done to ensure the safety of certain cosmetic products on human and not just limited to animal tests.

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