UPREGULATION OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR EXPRESSION BY OESTROGEN AND BAK FOONG PILL IN MOUSE UTERI

D. K. ROWLANDS¹, L. L. TSANG¹, Y. G. CUI¹, Y. W. CHUNG¹, L. N. CHAN¹, C. Q. LIU³, TONY JAMES² and H. C. CHAN¹

¹Epithelial Cell Biology Research Center, Department of Physiology, Chinese University of Hong Kong, Shatin, Hong Kong, ²Laboratory Animal Services Centre, Chinese University of Hong Kong, Shatin, Hong Kong, ³Shanghai Institute of Planned Parenthood Research, Shanghai, China, ⁴Nanjing Medical University, Nanjing, China

Received 9 January 2001; accepted 13 February 2001

Although cystic fibrosis transmembrane conductance regulator (CFTR) has been shown to be expressed in the female reproductive tract, its functional role in the uterus is not fully understood. The present study investigated a possible physiological role of CFTR by comparing the effects of 17β-oestradiol and Bak Foong Pill (BFP), an over-the-counter Chinese medicine used for centuries for the treatment of various gynaecological disorders, on uterus size and the expression of CFTR in the uterus of ovariectomised mice using RT-PCR. Treatment of ovariectomised mice with 17β-oestradiol (0.2 mg/kg, p.o.) for 12 days caused a significant increase in uterine wet weight compared to vehicle. However, treatment with BFP (3 g/kg, p.o.) for the same period failed to increase uterine wet weight, indicating a lack of direct oestrogen-like activity of BFP. Analysis of CFTR mRNA expression in the harvested uteri using RT-PCR showed that both 17β-oestradiol and BFP induced an increase in CFTR mRNA expression in mouse uteri compared to levels observed in vehicle-treated animals. These results suggest that CFTR can be upregulated by oestrogen and BFP, however, the effect exerted by BFP does not seem to be mediated by direct oestrogen-like activity. Regulation of CFTR expression by both oestrogen and gynaecological medication BFP indicates an important role of CFTR in reproductive functions.

KEYWORDS: CFTR; endometrium; mouse; RT-PCR; oestrogen.

INTRODUCTION

Cystic fibrosis conductance regulator (CFTR) is a cAMP-regulated Cl⁻ channel, mutations in which are found to be responsible for the disease cystic fibrosis (CF), which affects most of the exocrine glands and tissues including the reproductive tracts (Quinton, 1999). Expression of CFTR in female reproductive tracts of a number of species including humans has been observed (Tizzano et al., 1994; Hayslip et al., 1997; Trezise et al., 1993) and shown to be influenced by ovarian hormones (Rochwerger and Buchwald, 1993; Rochwerger et al., 1994; Mularoni et al., 1995). Reduced fertility rate has been reported in women with CF (Kopito et al., 1973), but the physiological role of CFTR remains largely unknown, although CFTR has been implicated as a cAMP-dependent chloride channel which might be responsible for salt and water transport across the endometrial epithelium thereby regulating the uterine fluid environment (Chan et al., 1999, Chan et al., 2000).
The present study investigated a possible physiological role of CFTR by comparing the effects of 17β-oestradiol and Bak Foong Pill (BFP), a traditional ‘over-the-counter’ Chinese medicine for the treatment of various gynaecological disorders, on uterus size and the expression of CFTR in the uterus of ovariectomised ICR mice. Recent clinical trials have demonstrated that the symptoms of women suffering of various gynaecological disorders were greatly improved when treated daily with BFP (300 mg/kg p.o.) for periods of 4–8 weeks (unpublished data). The reduction observed in irregular menstruation was especially prevalent, leading to suggestions that BFP had oestrogen-like activity. Since both the uterus size and CFTR expression are known to be influenced by oestrogen, a comparison of the effects of BFP to that of oestrogen may reveal the mechanism underlying BFP action and thus indicate the possible involvement of CFTR in normal and pathophysiological conditions.

MATERIALS AND METHODS

Treatment
Adult female ICR mice (40 g bodyweight) were bilaterally ovariectomised under ketamine (75 mg/kg i.p.) and xylasine (10 mg/kg i.p.) anaesthesia and left to recover for 3 weeks. Mice were then divided into groups of 10 and treated with either BFP [3 g/kg, purchased from Eu Yan Sang (Hong Kong) Ltd], 17β-oestradiol (0.2 mg/kg) or vehicle (0.4 ml dH₂O) via oral gavage daily for 12 days. Animals were then killed and their uteri removed, weighed and expressed as uterine wet weight/bodyweight (mg/kg) before freezing in liquid nitrogen and stored at −70°C until analysis. Statistical levels of significance were determined using one-way ANOVA followed by Newman-Keuls multiple comparison test.

Semi-quantitative RT-PCR
RNA extraction and RT-PCR were carried out within one week of uterine harvest. The specific oligo nucleotide primers for CFTR were: CAT CTT TGG TGT TTC CTA TGA TG (sense) and GTA AGG TCT CAG TTA GAA TTG AA (antisense), corresponding to nucleotide 566–1017 with expected cDNA of 452 bp. GAPDH was used as an internal standard with oligo nucleotide primers: ACC ACA GTC CAT GCC ATC AC (sense) and TCC ACC ACC CTG TTG CTG TA (antisense), corresponding to nucleotide 566–1017 with excepted cDNA of 452 bp.

RESULTS
Treatment of animals with 17β-oestradiol (0.2 mg/kg, p.o.) caused a significant increase in uterine wet weight compared to vehicle (0.274 ± 0.04% bodyweight and 0.10 ± 0.03% bodyweight respectively, n=9, P<0.01). Whereas, treatment with BFP (3 g/kg, p.o.) for the same period failed to increase uterine wet weight (0.097 ± 0.09% bodyweight, P>0.05, n=9) (Fig. 1). RT-PCR analysis demonstrated approximately a seven-fold increase in CFTR mRNA expression following 17β-oestradiol treatment and a three-fold increase following BFP treatment when compared to vehicle (Fig. 2).

DISCUSSION
The present study has demonstrated that CFTR can be upregulated by both oestrogen and BFP but through different mechanisms. Our findings that BFP failed to cause any increase in uterus size compared to vehicle, whereas treatment with 17β-oestradiol significantly increased uterus size above normal, demonstrate that BFP has no direct oestrogenic activity of its own. Conversely, our data using semi-quantitative RT-PCR has revealed that
oestrogen, as previously reported, causes increased expression of CFTR mRNA in mouse uteri (Rochwerger and Buchwald, 1993). However, despite BFP being unable to mimic oestrogen in causing an increase in uterus size, BFP was able to induce an increase in CFTR mRNA expression. This rather surprising finding suggests that increased CFTR mRNA expression induced by BFP may be caused by a different mechanism to that of oestrogen-stimulated CFTR expression. The fact that both oestrogen and BFP cause increases in CFTR expression may suggest that CFTR expression is an important determinant for normal uterine function and may be involved in mediating the beneficial effect of BFP in improving pathophysiological conditions.

ACKNOWLEDGEMENT

This work was supported by Innovation Technology Fund of Hong Kong.

REFERENCES


