PROTECTIVE EFFECT OF GBG1 ON THE MALE AND FEMALE REPRODUCTIVE SYSTEM DAMAGED BY TCDD AND ITS MECHANISM

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Introduction

In humans, the consequences of prenatal exposure to TCDD on the reproductive tract of both males and females have been identified and developmental neurological problems of TCDD in children are well known. Furthermore, many articles indicating declines in the quality and quantity of sperm production in humans over the last four decades, and increases in certain endocrine-related cancers have given speculation about environmental etiologies and development of protective agents against EDCs 2,3.

With all these reasons, this study focused on investigating the protective effects of GBG1, natural plant extract, on 2,3,7,8-tetrachlorodibenzo -p-dioxin(TCDD) induced male reproductive system damages and diethylstilbestrol induced female uterotrophic effect in animal models and its mechanism in in virto cell culture system.

Methods and Materials

(1) Animals

Male SD rats (4weeks old) were used.

(2) Materials

2,3,7,8-Tetrachlorodibenzo-p-dioxin(TCDD) (NIH, USA) was dissolved into minimum volume of acetone and diluted with corn oil. On first day of the second week, one dose of TCDD (15μg/kg /rat;intraperitoneal)was administered. GBG1 was disolved in distilled water and given 50mg/kg/day/rat through oral route.

(3) Measurement of accessory sex gland weight

Biopsy was perfomed 28 days after the TCDD injection. Prostate glands and seminal vesicles of rats from each group were weighted after removing fats

(4) Sperm motility

On removing left caudal epididymis (in anatomical position), we prepared sperm suspension solution. 200 sperms were counted to calculate motility ratio (%).

(5) Sperm head count

After removing and weighting the uncapsuled left testis, it was homogenized and sonicated. The homogenized sample was diluted, sperm heads were counted

(6) E-SCREEN Assay

E-SCREEN assay using MCF-7-BUS cells was carried out according to the Perez et al. (1998).

(7) Uterotrophic assay

Dienthylstilbestrol(DES,50μg/kg), tamoxifen(Tam,1mg/kg) were dissolved in saline and given

intraperitoneally. GBG1 also dissolved in saline and injected intraperitoneally for 3 days before DES treatment. Uterotrophic assay performed according to the OECD protocol.
(8) Statistics
For the data, t-tests were used to compare groups against control group. In all cases, $P < 0.05$ and $P < 0.01$ was considered statistically significant.

Results and Discussion

1. Protective effect of GBG1 on male reproductive system
(1) Change of body weight
The body weight change of TCDD treated group showed difference from that of control group in fourth and fifth week, however the difference was not meaningful.
(2) Weight of accessory sex glands
The ratio of prostate gland to body weight in TCDD treated group did not show significant change compared with control group. But the simultaneous administration of GBG1 showed the higher ratio of prostate gland to body weight than control group .
(3) Sperm head count
The TCDD treated group showed significant decrease compared with control group and all the group of GBG1 administration were recovered to the control level. Especially, the group to which GBG1 administered simultaneously with TCDD showed the significant increase of sperm number compared with the group of TCDD administration only ($p<0.01$).
(4) Sperm motility
The TCDD treated group showed significant decrease of motility ($p<0.01$) compared with the control group. The simultaneous administration of GBG1 group and the subsequent administration of GBG1 group showed significant recovery of 15.0% and 16.2%, respectively ($p<0.05$).

2. Action mechanism of GBG1
(1) Estrogenicity of GBG1
In order to quantify the estrogenic activity of GBG1 itself, various concentrations of GBG1 treated to MCF7-BUS cells for 6 days. There was no difference between control and GBG1 treated groups (0.001 - 1mg/L). From this reasult, we concluded that GBG1 itself has no estrogenic activity.
(2) Inhibitory effect of GBG1 on TCDD antiestrogenic activity.
17β -estradiol(10^{-11}M) showed estrogenic activity through MCF7-BUS cell proliferation and TCDD(10^{-10}M) showed antiestrogenic activity through the inhibitory effect on 17β -estradiol induced cell proliferation. Inhibitory effect of TCDD to 17β -estradiol induced cell proliferation was declined by GBG1(0.001-1mg/L) treatment with dose dependent manner.

3. Protective effect of GBG1 on female reproductive system
(1) Uterotrophic effect of GBG1
To confirm the uterotrophic effect of GBG1 itself, various does of GBG1 treated to the female 3 week old SD rats. Wet weight of uterus in DES treated group was 6 times higher than control group. But there was no difference GBG1 treated group(10mg-250mg/kg) and control group. From this result, we concluded that GBG1 itself has no uterotrophic effect.
(2) Inhibitory effect of GBG1 on DES uterotrophic activity
Tamoxifen, well known inhibitor to DES, effectively decreased the wet, dry weight of uterus in DES treated group. GBG1 moderately suppressed the increase of wet, dry weight of uterus in

DES treated group, but not significant.
So, we concluded that GBG1 has moderate inhibitory effect to DES uterotrophic activity.

References

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