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Short Communication

Antiandrogen Flutamide Might Protect Multi-Generations of Rats from Androgen-Dependent Toxicity in Spinal and Bulbar Muscular Atrophy Induced by Benomyl or Carbendazim

Shui-Yuan Lu* and Wei-Ren Tsai

Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taiwan, Republic of China

***Corresponding Author:** Shui-Yuan Lu, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Executive Yuan, Taichung, Taiwan, Republic of China.

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As we all knew that carbendazim is a systemic fungicide for crops [1]. Carbendazim and its parent precursor fungicide, benomyl (Figure 1), exhibited low acute toxicity with oral LD_{50} in male rats greater than 15 g/kg [2]. In contrary to the low acute toxicity, carbendazim and its parent compound benomyl showed reproductive toxicity in rodents [3-6]. Carbendazim and benomyl produced male reproductive toxicity, such as testicular disfunction by inducing the sloughing of germ cells [7,8], testicular toxicity [9], testicular lesion [10] and sterility [11].

In the respect of developmental toxicity, carbendazim and benomyl induced significant toxicity. Prenatal administration of carbendazim to rats during pregnancy produced exencephaly, microphthalmia and hydronephrosis in offspring [3,12]. Benomyl produced malformations such as cleft palate, exencephaly in prenatal developmental study [13]. In the co-treatment study with carbendazim and flutamide for 28 days, testicular weight, losses of spermatozoa concentration and cell morphology induced by carbendazim can be recovered by flutamide. Carbendazim induced androgen receptor related abnormalities including incomplete development of uterine horn, detention of urethra, disappearance of vagina and induction of extra seminal vesicles in female offsprings. Also, benomyl produced androgen receptor related malformations such as incomplete development, disappearance of vagina in female rats, testis and epididymis atrophy in male rats. Treatment with a longer duration, carbendazim induced androgen receptor expression with dose dependent manner in testis and epididymis. The *in vivo* results of androgen receptor expression was confirmed by carbendazim binding to androgen receptor with concentration dependent *in vitro* with testis extracts. We have showed that carbendazim-induced reproductive and developmental malformations are relieved by flutamide. This implied that androgen receptor-mediated reproductive and developmental toxicity induced by carbendazim [14]. Later, we further studied the endocrine disrupting endpoints with co-treatment carbendazim and flutamide to examine if the block effect will be significant. The results showed that the increased anogenital distance (AGD) in utero exposure carbendazim or benomyl in male pups were recovered by flutamide [15].

In order to prove that carbendazim-induced androgen receptor expression antagonized by flutamide in male rats we carried out the next study. We studied the androgen receptor expression in the mRNA and protein level with Western blot and activity with immunohistochemistry. Carbendazim induced androgen receptor expression of mRNA and protein with dose and time dependent and immune activity in testes of rats. All these endpoints can be recovered by flutamide treatment [16].

Though, anogenital distance is a representative endpoints of endocrine disrupting activity (EDA) so far two of standard protocol for endocrine disturb are uterotrophic assay (US EPA Series 890.1600) and Hershberger assay (US EPA Series 890.1400). We conduct these two protocols to study the expression of androgen receptor in a short-term treatment. The results showed that benomyl or carbendazim increased uterine weight in uterotrophic assay and weights of ventral prostate plus seminal vesicles through androgen receptor expression while flutamide can abolish the male accessory gland weight [17].

Based on above results we concluded that reproductive and developmental toxicity and endocrine disrupting activity induced by carbendazim or benomyl can be recovered by flutamide. Newly we found that carbendazim might induce spinal and bulbar muscular atrophy in multi-generation of rats [18].

Though we did not prove that flutamide can abolish the spinal and bulbar muscular atrophy in the multi-generation rats a report showed that prenatal flutamide enhances survival in a myogenic mouse model of spinal bulbar muscular atrophy [19]. Four years later, another report confirmed that antiandrogen flutamide protects male mice from androgen-dependent toxicity in three models of spinal bulbar muscular atrophy [20]. In contrary to this result two reports showed that flutamide did not abrogate the SBMA through AR mediated in mice [21,22]. So far there is no reason for the difference between them. In spite of the difference of flutamide action, linking antagonistic effects of carbendazim and flutamide exposure in utero on reproductive and developmental toxicity in rats and androgen receptor expression antagonized by flutamide in male rats we are confident in flutamide abrogating the SBMA with supporting by Johansen., *et al.* (2020) [19] and Renier., *et al.* (2014) [20].

Based on chemical structure activity relationship we hypothesis that carbendazim and benomyl exhibited androgen receptor (AR) agonistic effect by sharing C and D ring with dihydrotestosterone (DHT) (Figure 1) while flutamide showed AR antagonistic effect by sharing A ring with DHT referred to the previous report by Tamura., *et al*. (2003) [23].

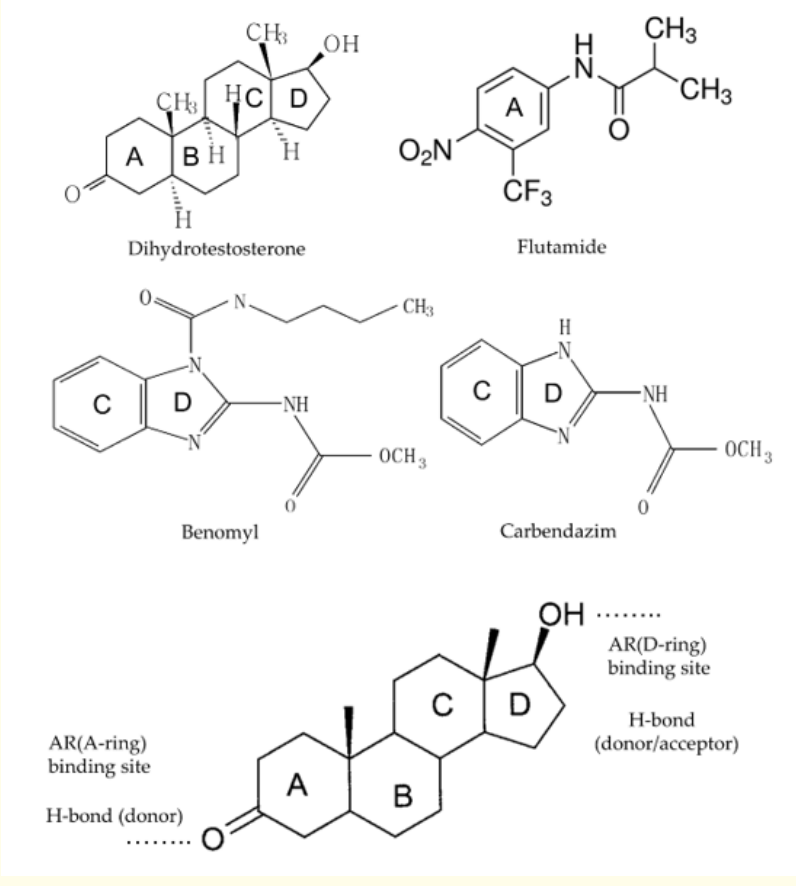

Figure 1: *Schematic labeling of the benomyl, carbendazim mimic the main ligand interaction features of the natural ligand, dihydrotestosterone, with the androgen receptor.*

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