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Moclobemide-induced gynecomastia in rats

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KEY WORDS moclobemide; toxicity; gynecomastia; prolactin; prolactin receptors; rat

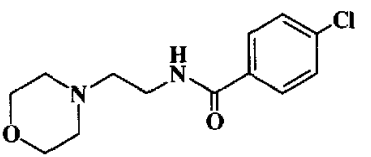
ABSTRACT

AIM: To study the toxic effect of moclobemide on male breast and to elucidate its mechanism of action. **METHODS**: Routine histopathological analysis was used to diagnose the effect of moclobemide on male breast in rats. Plasma concentrations of estrogen, androgen, and prolactin were measured by a ratioimmunometer and relative receptors of mammary gland tissue were detected immunohistochemically. **RESULTS**: After 180-d moclobemide treatment, the presence of gynecomastia was 0, 5, 5, 7/10 rats in 0, 60, 240, and 600 mg/kg groups, respectively. After 30-d convalescence, only one rat in 600 mg/kg group got the incidence of gynecomastia. Serum prolactin concentration had a trend to decrease with increasing dose and prolactin receptors in mammary gland were up-regulated. **CONCLUSION**: Long-term treatment with moclobemide causes gynecomastia in rats, which is reversible. The mechanism of moclobemide-induced gynecomastia may be related to the increase in prolactin receptors in mammary glands.

INTRODUCTION

Male breast carcinoma is rare, while the presentation of a male patient with evidence of breast enlargement or of a palpable lump is not uncommon[1]. It has been reported that approximately 85 % of male breast masses were due to gynecomastia[2]. Clinically, drug-induced mammary enlargement is more frequent than other reasons[3]. Disturbance in hormonal balance is regarded as an important reason for gynecomastia[4].

Moclobemide {4-chloro-*N*-[2-(4-morpholinyl)-ethyl] benzamide} is a novel inhibitor of the enzyme monoamine oxidase (MAO) distinguished by the reversibility of its action and its predominant effect on MAO-A[5]. Clinical trials have shown that the drug possesses antidepressant therapeutic efficacy comparable with that of some tricyclic drugs[6]. Moll *et al* concluded that it was safe and efficient during a long-term treatment[7].



However a number of reports suggest that moclobemide may cause hormonal inbalance as there is evidence that moclobemide increased prolactin secretion in healthy male volunteers[8]. In another study, long-term treatment with moclobemide increased cortisol secretion, but had no influence on growth hormone, prolactin, testosterone, luteinizing hormone or follicle-stimulating hormone[9]. In rats, moclobemide treatment significantly attenuated stress-induced plasma ACTH and corticosterone levels. Hippocampal mineralocorticoid receptor and glucocorticoid receptor levels were up-regulated[10].

A study also confirmed that moclobemide could be excreted in breast milk[11]. But there is no report documenting the effect of moclobemide on male breast in human or in experimental animals to date. The objective of the current investigation was to study the toxic effect of moclobemide on the male breast and to observe its reversibility.

MATERIALS AND METHODS

Drugs and reagents Moclobemide was supplied by *Shanghai XinYi Pharmaceutical Factory*. Antisera of hormones, antiserum of prolactin receptor and ABC immunodetection kits were obtained from Larvin, DoKa and Sino-American Biotechnology Co.

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Animal treatment Sixty-four male rats (Sprague Dawley, grade Ⅱ, Certificate № 02-49-2, 6 wk, 150.2 g ± 16.9 g, from Shanghai SIPPR-BK Co) were selected, divided into 4 groups, and treated with placebo, moclobemide 60, 240, or 600 mg/kg, respectively. Each group contained 16 rats, 10 of them underwent normal 180-d treatment and the rest were convalesced for other 30 d, a total of 210 d. The drug or placebo was given once a day at 8:30 am, *po*. Animal facilities were maintained under 12-h light/dark cycle (light on at 7:30 am), 22 ℃ ± 2 ℃ and 50 % – 70 % relative humidity. with food and water were available *ad lib*.

Routine analysis Blood and urine were collected for routine analysis on d 180 or d 210. Hematological examination was performed on a blood autoanalyzer (Cobas Minos Stel, Roche Co). Serum and urine chemistry was detected with a biochemistry autoanalyzer (Type 700, Beckman Co).

Systemic autopsy The rats were autopsied on d 180 or d 210. Heart, lungs, livers, spleen, kidneys, adrenals, stomach, testes, prostate, pituitary, and mammary glands were removed, examined, and organ coefficients were counted.

Histopathological analysis Each organ was fixed in 10 % formalin, embedded in paraffin, sectioned at 6 μm, stained with HE[12], and observed under light microscopy. Hyperplastic, fibrous, and an intermediate group with both hyperplastic and fibrous features was determined as gynecomastia[13].

Hormone detection Venous blood was collected before autopsy for plasma hormone detection. Specific antisera (Larvin Biotechnology Co) were used in detecting hormone concentrations and the detection was performed on a radioimmunometer (SN-695, Shanghai Institute of Nuclear Research, Chinese Academy of Sciences).

Immunohistochemical assay The experimental methodology of this assay is described previously[13]. Sections of mammary glands were stained with Avidin Biotin Complex (ABC), then measured at 580 nm wavelength with microspectrophotometer (MPV-SP, LEICA Co) to determine the prolactin receptor level by comparing absorbance (A) at 580 nm of treatment groups with the control group.

Statistics Data were processed with an analysis program EPI 5 (Public Domain Software for Epidemiology and Disease Surveillance). Within each group, a paired t test or chi-square test was used to determine whether there was a significant difference. Differences were considered statistically significant if the P value was less than 0.05 or 0.01.

RESULTS

Systemic examination After 180- or 210-d treatment, no significant changes were found in routine analyses of hematology, serum chemistry, and urine chemistry. Almost all organs: heart, lungs, livers, spleen, kidneys, adrenal, stomach, testes, prostate, and pituitary were in good condition and their organ coefficients were in normal ranges.

Mammary glands Histopathological analysis revealed that mammary gland developed and proliferated in 180-d moclobemide-treated groups. The presence of gynecomastia was 50 % in 60 and 240 mg/kg groups, 70 % in 600 mg/kg group (Tab 1). After 30-d convalescence, the incidence of gynecomastia decreased significantly in 600 mg/kg group and disappeared in 60 and 240 mg/kg groups. The proliferated mammary glands and mammary glands in control group are shown in Fig 1 A and C.

Tab 1. Effect of moclobemide on male breast in rats. AD: A 180-d treatment with moclobemide or placebo; WD: 30-d convalescence after 180-d treatment. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Dosage (mg/kg)	Administration (AD) 0	60	240	600	Withdrawal (WD) 0	60	240	600
Rats (n)	10	10	10	10	6	6	6	6
Hyperplasia (n)	0	5[b]	5[b]	7[c]	0	0	0	1

Prolactin receptor The mammary prolactin receptor level increased significantly in moclobemide-treated (240 and 600 mg/kg) groups, and recovered after 30-d convalescence (Tab 2). The immunochemically stained mammary glands are shown in Fig 1 B and D.

Hormone detection No change in the levels of growth hormone, estrogen, testosterone, luteinizing hormone or follicle-stimulating hormone was found. The ratio of estradiol and testosterone was not affected as compared with the control. Serum prolactin concentration had a trend (Tab 3) to decrease in moclobemide-treated groups and was observed to be recovered after convalescence.

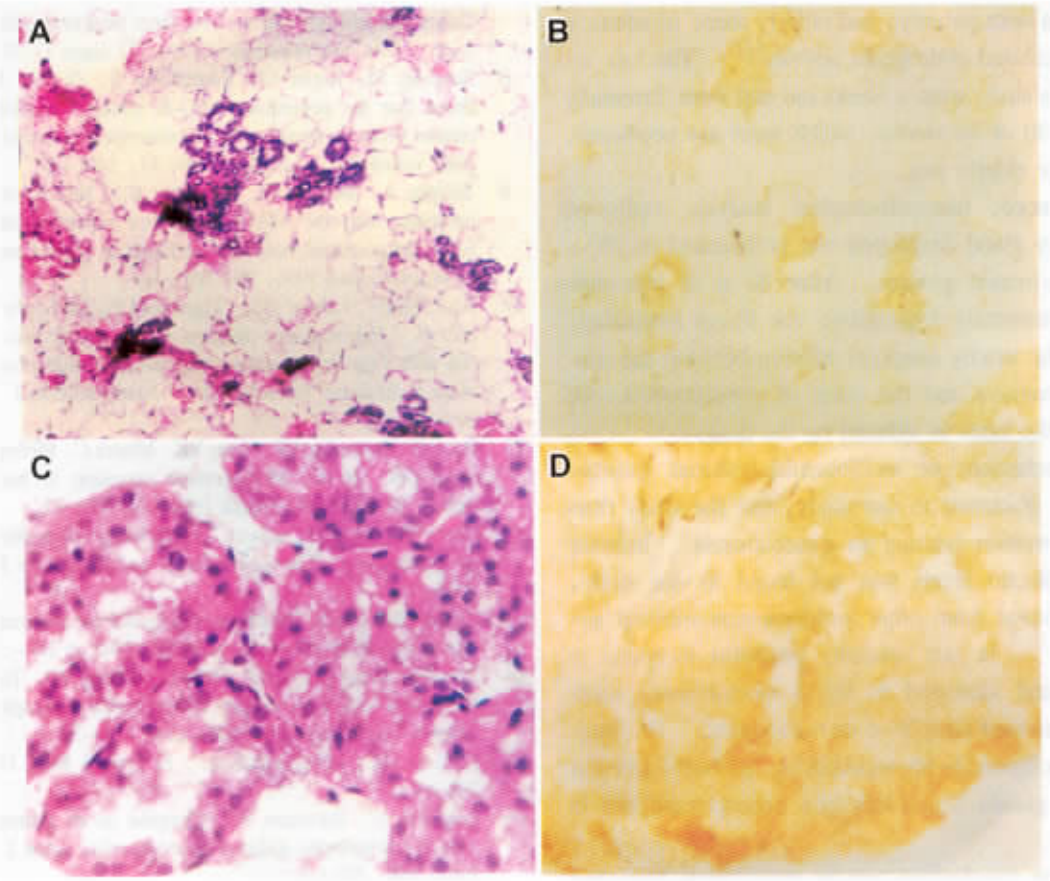

Fig 1. Histopathological analysis (A and C) of mammary glands in rats. Immunohistochemical staining (B and D) for prolactin receptor in mammary gland of male rats. (A) Control group placebo-treated for 180 d shows thick fatty tissues, few mammary lobes and intralobular ducts. ×33. (B) The mammary tissue shows weak staining for prolactin receptors in placebo-treated group for 180 d. ×33. (C) 600 mg/kg group moclobemide-treated for 180 d shows richly proliferated alveoli. ×132. (D) The mammary tissue shows strong staining for prolactin receptors in 600 mg/kg group for 180 d. ×132.

Tab 2. Effects of moclobemide on mammary prolactin receptors in male rats. AD: A 180-d treatment with moclobemide or placebo, $n = 50$; WD: 30-d convalescence after 180-d treatment, $n = 30$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Dosage (mg/kg)	Administration (AD) $A_{580\ nm}$	Withdrawal (WD) $A_{580\ nm}$
0	0.10 ± 0.03	0.099 ± 0.020
60	0.10 ± 0.03	0.10 ± 0.03
240	0.12 ± 0.04^b	0.11 ± 0.05
600	0.13 ± 0.05^c	0.12 ± 0.06

Tab 3. Effects of moclobemide on serum prolactin in male rats. AD: A 180-d treatment with moclobemide or placebo, $n = 10$; WD: 30-d convalescence after 180-d treatment, $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

Dosage (mg/kg)	Administration (AD) μg/L	Withdrawal (WD) μg/L
0	5.19 ± 0.26	4.99 ± 0.31
60	4.73 ± 0.30	4.55 ± 0.33
240	4.61 ± 0.21	4.08 ± 0.21
600	4.13 ± 0.33	3.82 ± 0.25^b

DISCUSSION

Clinical conditions associated with gynecomastia have been summarized by Carlson[15]: hypogonadism; neoplasm, such as adrenal tumors and tumors of the Leydig cells of the testis; systemic diseases, such as renal failure and hepatic disorders; drug-induced gynecomastia, etc. In our study, disorders of livers, kidneys, testes, pituitary, and adrenal were not found and gynecomastia associated clinical conditions were excluded.

Gynecomastia is normal in three groups: newborns, in whom it is transient due to exposure to maternal estrogens; adolescents, who experience a transient condition

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in association with puberty; and elderly men, in whom it is due to decreased androgenic activity[4]. The rats selected for this study were 6 weeks old and were terminally examined at 30 or 34 weeks, which were not newborns, adolescents or elderly rats.

Furthermore, histopathological analysis confirmed that mammary gland developed and proliferated in 180-d moclobemide-treated groups. After 30 d of convalescence, few mammary hyperplasia was found remaining. Because of the strictly temporal relation between the moclobemide treatment and the onset of gynecomastia, we suggest that the latter is induced by the drug.

The mechanism of moclobemide-induced gynecomastia is not discussed in our study, but the study does provide information relating to gynecomastia. Increase in serum prolactin levels was not found in our study, which is different from other antidepressant-induced gynecomastia[16]. In our opinion, prolactin receptors in mammary gland increased in 180-d moclobemide treatment and recovered after 30-d convalescence. This suggests that moclobemide up-regulates the prolactin receptor in mammary gland, which may play an important role in gynecomastia.

Following conclusions can be thus drawn from this study: long-term treatment with moclobemide causes gynecomastia in rats, which is reversible. The mechanism of moclobemide-induced gynecomastia may be related to an increase in prolactin receptors in mammary glands. To our knowledge this is the first study documenting moclobemide-induced gynecomastia. It is necessary to study further the molecular and clinical toxicity of moclobemide with regard to the male breast.

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吗氯贝胺源性雄鼠乳腺发育症

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关键词 吗氯贝胺; 毒性; 男子乳腺发育; 催乳素; 催乳素受体; 大鼠

目的: 研究吗氯贝胺的乳腺毒性及其可能机制. **方法**: 用组织病理学方法判断吗氯贝胺对乳腺的影响, 用免疫荧光的方法检测雄激素、雌激素和催乳素的血清浓度, 并用免疫组织化学方法半定量测定相关受体的变化. **结果**: 180 d 时对照组、60、240、600 mg/kg 给药组分别有 0、5、5、7 只大鼠乳腺发育, 30 d 恢复期后仅 600 mg/kg 组有 1 只大鼠乳腺发育. 血清催乳素的浓度随着给药剂量的升高而下降, 乳腺组织催乳素受体上调. **结论**: 长期服用吗氯贝胺可引起可逆性雄性大鼠乳腺发育, 异常发育的机制可能与催乳素受体上调有关.

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