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# Inhibition by Hydroxy-*N*-methyl Elliptinium of Precancerous Mammary Hyperplastic Alveolar Nodule Formation in Mice*

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**Abstract**—*Daily subcutaneous injections of* 5 μg *hydroxy-*N*-methyl elliptinium for* 4 weeks *in a high mammary tumor strain of SHN virgin mice was found to decrease the number of precancerous mammary hyperplastic alveolar nodules (HAN) associated with the increase in the number of ghosts, the remnants of regressed HAN. On the other hand, the treatment had little influence on the normal mammary lobulo-alveolar system, serum prolactin level, estrous cycle and endocrine organ weights. The results have demonstrated that elliptinium can inhibit the formation of HAN and induce their regression with no modulation of endogenous conditions of mammotropic hormones.*

## INTRODUCTION

THERE have been several articles written about the anticancer properties of elliptinium in human cancers involving breast cancer [1–4] and on its pharmacokinetics in human [5] and mice [6]. However, no data are available on the basic studies of the effects of this drug on the animal tumor models except for that on L1210, a transplantable mouse leukemia [7].

In this paper we report the inhibition by elliptinium of mammary hyperplastic alveolar nodules (HAN), a precancerous state of spontaneous mammary tumors in mice [8], as a possible step to obtain fundamental information on the mechanism of the anticancer role of this drug in breast cancer. Its effect on the circulating prolactin was also studied, since prolactin is a primary hormone for development and progression of HAN [9].

## MATERIALS AND METHODS

*Animals*

A high mammary tumor strain of SHN virgin mice bred in the authors' laboratory were used. At 6 months of age the experimental mice received daily subcutaneous injections of 5 μg hydroxy-*N*-methyl elliptinium (SANOFI, Paris, France: Lot 181 003) suspended in 0.05 ml physiologic saline for 4 weeks. The control mice were given the vehicle only. On the day following the last injection all the mice were killed by decapitation.

Throughout the experiment, mice were kept in Teflon cages (18 × 30 × 15 cm) with wood shavings, 5 mice in each, maintained in an animal room that was air-conditioned (23 ± 0.5°C and 65–75% relative humidity) and artificially illuminated (14 hr of light, from 5:00 a.m. to 7:00 p.m.), and provided with a commercial diet (CE-2: CLEA Japan, Tokyo, Japan) and tap water *ad libitum*.

*Normal and preneoplastic mammary gland growth*

At autopsy, bilateral thoracic third mammary glands were prepared for whole-mount evaluation and checked at ×10 magnification. The degree of growth of normal lobulo-alveolar system was rated from 1 to 7 in increments of 1 [10], and the mean of the rating in the bilateral glands was employed for the representative value for each animal. The number of HAN was counted and the value for each mouse was expressed in terms of the sum of the numbers in the bilateral glands. The mathematical mean of the major two diameters of each HAN was also calculated as an index of the size. Furthermore,

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the number of 'ghosts', the remnants of regressed HAN, was counted.

*Serum prolactin levels*

Blood was collected from the trunk in 10 mice in each group at 0:00–1:00 p.m. on the day of autopsy. Serum prolactin level was assayed by the homologous radioimmunoassay using the kit donated by NIH, U.S.A.

*Body and endocrine organ weights*

All mice were weighed at the beginning of injection and just before autopsy. Anterior pituitary, adrenals and ovaries were immediately removed and weighed at autopsy in 10 mice from each group.

*Estrous cycle*

Throughout the experiment, each mouse had vaginal smears taken every morning (9:30–10:00 a.m.). The average length in days and the percentage of each stage of the cycle were calculated.

*Statistics*

The statistical significance of difference between groups in each parameter was evaluated by the Student's $t$ test.

## RESULTS

*Normal and precancerous mammary gland growth (Fig. 1)*

The number of HAN was significantly lower ($P<0.01$) and that of ghosts was higher ($P<0.05$) in the experimental mice than in the control. The size of HAN, which had large variations, was little different between the groups (data not shown).

Meanwhile, no difference was seen between groups in mammary rating as an index of normal lobulo-alveolar formation.

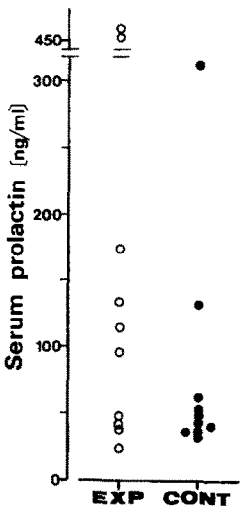


*Fig. 2. Serum prolactin levels in each group (means ± S.E.M.). See Fig. 1 for details of the treatment.*

*Serum prolactin level (Fig. 2)*

There is little difference between the experimental and the control mice in serum prolactin level. High levels observed in some mice of each group may partly be due to the continued estrous and metestrous stages of the estrous cycle (Table 1).

*Body and endocrine organ weights (Table 2)*

While there was little difference between groups in the body weight at the beginning of injection (initial), the weight at autopsy (final) was significantly higher in the experimental mice than in the controls ($P<0.05$). Moreover, the percentage change in body weight during the experiment was significantly higher than zero only in the experimental group ($P<0.001$).

No difference between the groups was found in any endocrine organ weight examined.

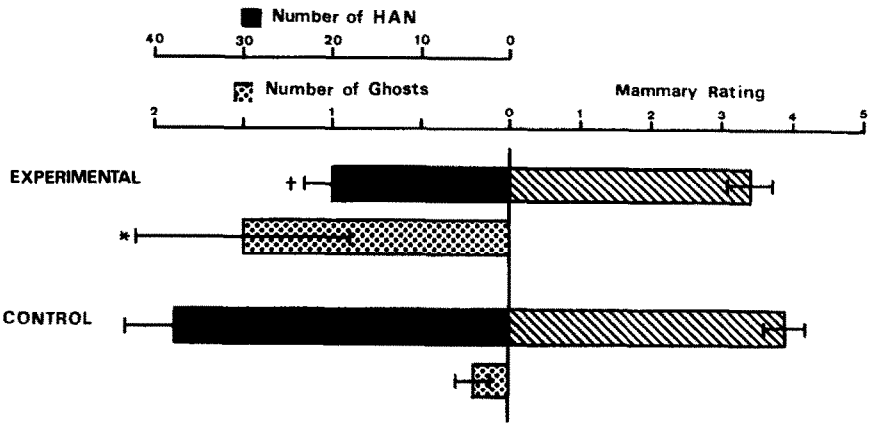


*Fig. 1. Normal and preneoplastic mammary gland growth in each group (mean ± S.E.M.). Experimental mice were given daily subcutaneous injections of 5 μg elliptinium for 4 weeks. The control received the vehicle only. The number of mice used was 20 in each group. * and †: significantly different from the control at $P<0.05$ and 0.001 respectively.*

*Table* 1. *Length of each stage of the estrous cycle in each group during the* 4 weeks *of examination (mean ± S.E.M.)*

| Group and treatment* | | Total periods examined | Proestrus | Estrus | Metestrus | Diestrus |
|---|---|---|---|---|---|---|
| Experimental | Days | 28 | 0.5 ± 0.2 | 8.6 ± 1.7 | 10.1 ± 2.4 | 7.6 ± 2.0 |
| (11)† | % | 100 | 1.7 ± 0.3 | 31.9 ± 6.2 | 38.1 ± 9.9 | 28.3 ± 7.4 |
| Control | Days | 28 | 0.7 ± 0.2 | 10.1 ± 1.9 | 9.1 ± 0.3 | 7.5 ± 2.3 |
| (11) | % | 100 | 2.7 ± 0.9 | 37.7 ± 7.0 | 32.4 ± 8.3 | 27.8 ± 8.0 |

*See Figure 1 for details of the treatment.
†Number of mice examined.

*Table* 2. *Body weight change and endocrine organ weights in each group (mean ± S.E.M.)*

| Group and treatment* | No. of mice | Body weight change Initial (g) | Final (g) | % change | No. of samples | Organ weight (mg) Anterior pituitary | Adrenals | Ovaries |
|---|---|---|---|---|---|---|---|---|
| Experimental | 20 | 30.3 ± 0.3 | 32.0 ± 0.5† | 6.0 ± 1.5‡ | 10 | 2.62 ± 0.24 | 21.5 ± 1.8 | 11.3 ± 0.6 |
| Control | 20 | 29.9 ± 0.5 | 30.7 ± 0.5 | 3.1 ± 1.9 | 10 | 2.60 ± 0.26 | 24.0 ± 1.5 | 12.7 ± 0.4 |

*See Figure 1 for details of the treatment.
†Significantly different from the control at $P < 0.05$.
‡Significantly different from zero at $P < 0.001$.

*Estrous cycle* (*Table* 1)

Little difference between the groups was observed in the period of each stage and its percentage and, therefore, in the pattern of estrous cycles. Although there were large individual variations, most animals in each group had rather steady estrous and metestrous stages.

## DISCUSSION

The results show that daily injections of hydroxy-*N*-methyl elliptinium significantly decreased the number of HAN associated with the increase in the number of ghosts. They have demonstrated that elliptinium can inhibit the formation of precancerous mammary hyperplastic alveolar nodules and induce their regression in mice. They further indicate that this inhibitory role of elliptinium is not mediated by its modulation of endogenous conditions of mammotropic hormones, since none of the serum prolactin level, the pattern of estrous cycle and the organ weight was affected by elliptinium injection. This was also supported by the finding that the degree of normal lobuloalveolar formation in the experimental mice was similar to that of the control. If elliptinium effects were mediated by its regulation of mammotropic hormone secretion, a normal mammary gland system would also be affected by the treatment, as observed in CB-154. CB-154, which inhibits markedly HAN formation through its suppression of pituitary prolactin release, always accompanies the regression of normal mammary gland [11].

All of the SHN virgin mice developed HAN after 5 months of age, although the number varied and, therefore, the incidence was 100% in both experimental and control groups in this study, in which 6 months-old mice were used. Thus the number of HAN and ghosts in the bilateral third thoracic mammary glands were compared between groups as the index of the anticancer effect of the drug.

In any case, the present results of selective inhibition by elliptinium of HAN with little influence on normal mammary glands and its increase in the body weight appraise the value of this drug as an antitumor agent.

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