

RESTORATION BY MOTHERWORT (*Leonurus sibiricus* L) OF LACTATION SUPPRESSED BY PREGNANCY-DEPENDENT MAMMARY TUMORS IN GR/A MICE

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Summary

As a possible step to evaluate in the mammary gland the role of motherwort (*Leonurus sibiricus* L), a representative medicinal plant used traditionally for the therapy of gynecologic diseases, the effects of the agent on lactation which was suppressed by pregnancy-dependent mammary tumors (PDMT) were studied in GR/A mice. Beginning the day of placing with males at 45-50 days of age, female mice were given 60% methanol-extract of the aerial part of motherwort as drinking water at the concentration of 0.5% throughout the experiment. Mice developing PDMT during pregnancy [PDMT(+)] and given motherwort were similar to mice developing no PDMT [PDMT(-)] with or without motherwort treatment and were significantly higher than PDMT(+) mice given tap water in litter growth and mammary RNA/DNA ratio on day 12 of the 2nd lactation. Mammary DNA and RNA contents were also elevated by motherwort in PDMT(+) mice. The results suggest that motherwort can ameliorate lactation suppressed by PDMT through its stimulation of both growth and function of the mammary glands.

(Key Words: GR/A, Lactation, Motherwort, Mice, Pregnancy-Dependent Mammary Tumors).

Introduction

GR/A mice are characterized by developing pregnancy-dependent mammary tumors (PDMT). PDMT appear after the middle of each pregnancy, show maximal sizes at the end of pregnancy and disappear immediately after parturition regardless of lactation (Yanai and Nagasawa, 1978). We have found that the appearance of PDMT at the end of pregnancy has aftereffects on the subsequent lactation (Nagasawa et al., 1989). Litter growth on day 12 of lactation of mice

which developed PDMT was significantly lower than that of mice without PDMT. This was associated with lower mammary contents of DNA and RNA.

Recently, the significance of medicinal plants has been recognized again in Japan. Motherwort (*Leonurus sibiricus* L) is widely used as one of the most important medicinal plants for the therapy of gynecological disorders as well as an edible plant. However, like other medicinal plants, the fundamental study on the effects of this agent is lacking. As a possible step to clarify the role of motherwort in mammary glands, we examined its effects on lactation of GR/A mice which was declined by PDMT.

Materials and Methods

Animals and treatments

Female mice of the highly inbred GR/A strain were placed with males at 45-50 days of age and were simultaneously given 60% methanol extract of aerial part of motherwort (Nagasawa et al., 1990a) as drinking water at the concentration

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of 0.5% (5g/l tap water) throughout the experiment. Pregnant mice were kept individually and placed again with males only near parturition to induce concurrent pregnancy. Litter size was adjusted to 5-6 on the day of parturition (day 0 of lactation) and pups were nursed until day 20 of lactation when weaned. Only mice that delivered concurrently at the 2nd parity were used in this experiment. Incidences of PDMT were 10% and 60% at the 1st and the 2nd parities, respectively. The high incidence of PDMT during the 2nd pregnancy is why we used mice in the 2nd lactation. Mice were divided into two groups according to the presence of PDMT, which was checked by palpation, at the end of the 2nd pregnancy [PDMT(-) and PDMT(+)]. On the day of the 2nd parturition, litter size was adjusted to 5-6 again, weighed and nursed. After each litter was weighed on the morning (8:00-9:00 AM) of day 12, mice were killed by decapitation under the light ether anesthesia. All procedures were the same as detailed previously (Nagasawa et al., 1989).

Throughout the experiment, mice were kept in plastic cages (16 × 28 × 13 cm) with wood shavings, maintained in an animal room, which was air-conditioned (22-24 °C and 55-70% relative humidity) and artificially illuminated (14 hours of light from 5:00 AM to 7:00 PM) and provided with a commercial diet (Lab MR Breeder: Nihon Nosan Kyogo KK, Yokohama, Japan) and water ad libitum.

The data of PDMT(-) and (+) mice giving tap water were cited from our previous paper (Nagasawa et al., 1989), since the set up of the same groups in this study was only the repetition, which is quite against the policy of animal welfare.

Reproduction

Delivery interval, litter size, still-birth rate, rate of still-born pups and rearing rate on day 12 were employed as the indices of reproduction (Nagasawa and Furukoshi, 1985).

Lactational performance

Average pup weight and % change in pup weight or pup growth rate on day 12 were employed as the indices of lactational performance.

Mammary nucleic acid contents

At autopsy, the unilateral inguinal glands which developed no PDMT were removed, defatted and dried with hot alcohol-ether. DNA and RNA were extracted by trichloroacetic acid and determined by diphenylamine and orcinol reactions, respectively, as described previously (Nagasawa and Yanai, 1974).

Endocrine organ weights

Anterior pituitary, adrenals and ovaries were weighed at autopsy and adrenals and ovaries were further examined histologically.

Plasma hormone levels

Blood was drawn into the heparinized tube, centrifuged at 1000 Xg for 20 min at 4°C and stored at -20°C. Plasma levels of prolactin and progesterone were determined by rat Nb₂ lymphoma cell bioassay (Lawson et al., 1982) and radioimmunoassay (De Villa et al., 1972), respectively.

Statistics

All parameters were expressed in terms of mean ± SEM and the statistical significance of difference between groups was evaluated by Duncan's multiple range test.

Results

Lactational performance and mammary nucleic acid contents (figure 1).

No difference was observed in any parameter examined between PDMT(-) mice given motherwort and those receiving tap water. Meanwhile, both weight and growth rate of pups and mammary RNA/DNA ratio of PDMT(+) mice receiving motherwort were elevated to the level of PDMT(-) mice and were significantly higher than those of PDMT(+) mice given tap water. PDMT(+) group given motherwort was also higher than those receiving tap water in mammary contents of DNA and RNA, although the differences were not statistically significant.

Reproduction (table 1)

There was no difference between groups in any parameter as the index of reproductivity.

Endocrine organ weights (table 2) and histological observation

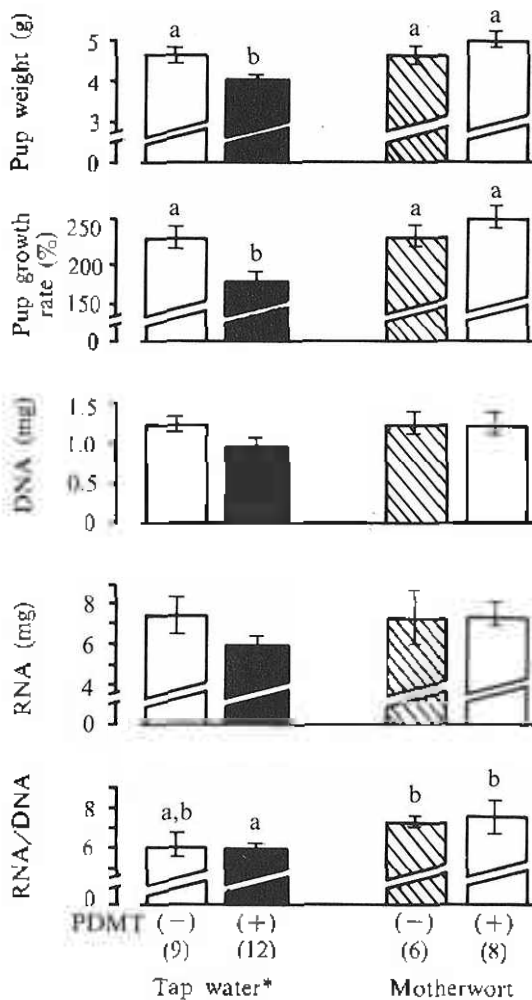


Figure 1. Effects of motherwort on lactation suppressed by pregnancy-dependent mammary tumors (PDMT) in GR/A mice. *From Nagasawa et al. 1989 with permission. Number of mice used is in the parentheses. ^{a,b}Values with different superscripts are different significantly at $p < 0.05$ or 0.01 .

While the adrenal weight was significantly lower in PDMT(+) group than in PDMT(-) group, slight differences were found between groups in the weights of anterior pituitary and ovaries. There was no difference between groups in the structure of adrenals and ovaries.

Plasma hormone levels (table 2)

Little difference was observed between groups

in the circulating level of either prolactin or progesterone.

Discussion

While lactation was suppressed by the presence of PDMT at the end of pregnancy (Nagasawa et al., 1989), there was no difference in any parameter as the index of lactational performance between PDMT(+) and PDMT(-) mice given motherwort in this study. Meanwhile, all values in PDMT(-) mice given motherwort were quite similar to those in PDMT(-) mice receiving tap water. The results indicate that, while motherwort shows little effects on lactation under normal conditions, it can ameliorate lactation suppressed by PDMT. The recovery by motherwort of lactation in PDMT(+) mice was further confirmed by the fact that all parameters were apparently higher in PDMT(+) mice given motherwort than in PDMT(+) mice receiving tap water.

Suppressed lactation of PDMT(+) mice was mostly due to the retarded growth of their mammary glands (Nagasawa et al., 1989). In this study, mammary RNA/DNA ratio of PDMT(+) mice was elevated to the level of PDMT(-) mice by motherwort treatment. The results indicate that the restoration of lactation by motherwort is principally attributed to its stimulation of mammary epithelial cell function. Furthermore, mammary DNA and RNA contents were also increased by motherwort in PDMT(+) mice. This suggests that the agent also contributes to the improvement of mammary cell growth. The mechanism of amelioration by motherwort of lactation is not clear at present. However, its involvement in the efficient utilization of some nutrients necessary for the growth and milk synthesis of the glands is suggested, since glucose tolerance is enhanced by motherwort (Nagasawa et al., 1990b). The present study revealed that motherwort had little effects on the circulating level of prolactin or progesterone and weights and/or histological structure of endocrine organs. Moreover, treatments with estrogen or progesterone and pituitary grafting singly or in combination influence neither litter growth nor mammary nucleic acid contents in PDMT(+) mice (Nagasawa and Suzuki, unpublished). These observations suggest that the involvement of endocrine system is minor in the process of the

TABLE 1. EFFECTS OF MOTHERWORT ON REPRODUCTION (Mean \pm SEM)

Group*	PDMT (-)	PDMT (+)
Number of mice	6	8
Delivery interval (days)	21.8 \pm 0.4	21.8 \pm 0.5
Litter size	8.2 \pm 0.6	6.5 \pm 0.8
Mother weight at parturition (g)	29.2 \pm 0.4	29.1 \pm 0.6
Still-birth rate (%)	0	0
Rate of still-born pups (%)	0	0
Rearing rate (%)	100	100

* Both groups received motherwort as drinking water (0.5%) throughout the experiment.

TABLE 2. EFFECTS OF MOTHERWORT ON ENDOCRINE ORGAN WEIGHTS AND PLASMA HORMONE LEVELS (Mean \pm SEM)

Group*	PDMT (-)	PDMT (+)
Number of mice	6	8
Final body weight (g)	35.1 \pm 0.8	34.1 \pm 0.6
Endocrine organ weight (mg)		
Anterior pituitary	4.0 \pm 0.3	3.5 \pm 0.2
Adrenals	6.7 \pm 0.1	5.9 \pm 0.1 ^a
Ovaries	13.3 \pm 0.6	12.1 \pm 0.4
Plasma hormone level (ng/ml)		
Prolactin	69 \pm 26	130 \pm 67
Progesterone	26.1 \pm 4.2	21.0 \pm 1.6

* Both groups received motherwort as drinking water (0.5%) throughout the experiment.

^aSignificantly smaller than PDMT(-) group at $p < 0.01$.

stimulation by motherwort of mammary gland growth and function.

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