Original Contribution

Inhibition of Spontaneous Mammary Tumourigenesis in Mice by Irradiation with Far-infrared Rays and the Effects of Bamboo Grass Leaf Extract on Tumour Growth

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Abstract: Mammary tumourigenesis in a high mammary tumour strain of SHN mice was inhibited significantly by exposure to FIR after 5 months of age, but not by FIR treatment between 1 month and 5 months of age. On the other hand, the formation of preneoplastic nodules was suppressed only by FIR irradiation between 1 month and 5 months of age, indicating that there is a critical period for FIR to inhibit the preneoplastic and neoplastic growth of the mammary glands. The partial inhibition of tumour growth caused by exposure to FIR for both periods was markedly enhanced by the combined administration of bamboo grass leaf extract, an active antitumour agent, in drinking water. The motor activity at 10 months of age was enhanced by FIR irradiation. FIR induced a significant elongation of life span. The findings indicate that an appropriate application of FIR would have beneficial effects at an advanced age.

Key Words: far-infrared rays, life span, mammary tumour, mice.

Introduction

Far-infrared ray (FIR) is attracting much attention with regard to applications in health and fitness. We have found that continued FIR irradiation after birth resulted in an elongation of life span in mice. However, the treatment had little effect on the prevention of spontaneous mammary tumours. The benefits of FIR in the hyperthermia of cancers have been reported both clinically and experimentally, especially in combination with chemotherapy and other treatments. Herein, we studied the effects of a shorter exposure to FIR on the development and growth of mammary tumours in SHN mice. The role of extract from bamboo grass leaves (Sasa Health®), a marked antitumour agent, in the growth of tumours was also examined.

Materials and Methods

Sample

Sasa Health® (Daiwa Biological Research Institute, Co., Ltd., Kawasaki, Japan), an extract of the leaves of bamboo grass (Sasa senanensis rehder) obtained by alkaline hydrolysis, was used. It was diluted 2.96-fold with tap water (0.088% Fe-chlorophyllin Na).

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Animals
A high mammary tumour strain of SHN/Mei female mice maintained in our laboratory by strict brother x sister matings was used. The animals were kept in aluminium cages (18×30×15 cm) with wood shavings (M size: CLEA JAPAN, Tokyo, Japan), 4-5 per cage, maintained in a windowless animal room, which was air-conditioned (24-25°C and 55-75% relative humidity) and artificially illuminated (14 h light cycle from 0500 to 1900 h), and provided with a commercial diet (Lab MR Breeder: Nihon Nosan Kogyo KK, Yokohama, Japan) and tap water ad libitum.

Procedures for all animals were carried out according to the USA NIH Guide for the Care and Use of Laboratory Animals.

Mammary tumour development
All mice were checked for palpable mammary tumours twice a week beginning at 3 months of age.

Mammary tumour growth
Beginning the day when the tumour reached 5-7 mm in size, expressed in terms of the geometric mean of the two major diameters (day 0), tumour size was recorded every 4 days until day 16. Procedures for the measurement were reported previously.

Mice in each group were divided into one of three categories according to the mammary tumour growth rate on day 16 (<100%, 100-200% and >200%).

Treatments
Three groups, one control group and two experimental groups, were set up. The control group was born and kept throughout the experiment on the normal rack. Experimental groups A and B were kept on the normal and FIR racks between 1 month and 5 months of age, respectively, and racks were changed thereafter.

To examine the combined effects of FIR and Sasa Health® on mammary tumour growth, some mice of groups A and B were given the agent in drinking water (0.088% Fe-chlorophyllin Na) after day 0 (experimental groups A + S and B + S, respectively).

FIR irradiation
The conditions were the same as detailed previously. The FIR source was positioned approximately 40 cm above the mice. This radiant heat panel was constructed from an Al₂O₃·TiO₂ ceramic-coated sheet of aluminium. The opposite side of the sheet was warmed by an electric heater, a laminate made from elemental carbon/polymer plastic composite material (Misato Plaheat Mfg., Ltd., Satte-shi, Japan). FIR emitted from ceramic ranged from 4 to 25 μm with a maximal intensity of 8 to 10 μm. Panel surface temperature was controlled at 40°C. An epoxy-coated-copper black ball (globe thermometer 75MM; Sibata Co., Ltd., Tokyo, Japan), a sensor (resistans Bulbs R 35; Ishikawa Seisakujo, Tokyo, Japan) and a recorder (μR1000 436002; Yokogawa Electric Co., Tokyo, Japan) constantly measured and recorded the temperature in the globe (effective temperature), which were 24-25°C and 26-27°C in the control and FIR racks (40 cm below the heat panel), respectively.
Spontaneous motor activity

At 6 and 10 months of age, spontaneous motor activity was measured in experimental groups A and B by Supermex (Muromachi Kikai Co., Ltd., Tokyo, Japan)\(^\text{16}\), a sensor monitor which was mounted above each cage to detect changes in heat across multiple zones of the cage through an array of Fresnel lenses. The body heat radiated by an animal was detected with paired IR pyroelectric detectors on the sensor head of the monitor. The system could monitor and count all spontaneous movements, both vertical and horizontal, including locomotion, rearing, head-movement, etc. All counts were automatically totaled and recorded at an hourly interval.

Life span

Each mouse was checked for health related conditions everyday and palpated twice a week for mammary tumour. Mice which were moribund or developed mammary tumours were killed under light ether anaesthesia immediately and in a week, respectively.

Survival rate at each month was obtained from the ratio of the number of survivors until that month against the initial number of mice examined.

Growth of normal and preneoplastic mammary glands

After recording the last size of mammary tumours on day 16, mice were killed by decapitation under light ether anaesthesia. The unilateral third thoracic glands were prepared for wholemount evaluation and examined under 10-fold magnification. The degree of normal end-bud formation was rated from 1-7 in increments of 1\(^\text{17}\). The number of preneoplastic mammary hyperplastic alveolar nodules (HAN) was counted and the area of each HAN was measured automatically by the computerized digitizer (Model LA-535: PIAS, Tokyo, Japan).

Weights of carcass and endocrine organs

At autopsy, carcass weight deducted mammary tumour weight from whole body weight was recorded. Anterior pituitary, adrenals and ovaries were removed and weighed.

Statistics

The significance of differences between the control and the experimental groups in mammary tumourigenesis and life span were evaluated by the two-way classification method of analysis of variance\(^\text{18}\). The difference in the other parameters between the groups was evaluated by Student's \(t\)-test.

Results

1. Mammary tumour development

Fig. 1 shows the cumulative incidence of mammary tumours in each group. The control and experimental groups A and B developed the first tumours at 6, 5 and 6 months of age, respectively. In group A relative to the control and group B between 7 and 12 months of age, the mammary tumourigenesis was significantly less severe considering simultaneously the incidence and the onset age of tumours.
2. Mammary tumour growth

As shown in Fig. 2, mammary tumour growth rate on day 16 was lower in both groups A and B than the control; however, the difference was not significant.

The combined treatment with Sasa Health® (groups A + S and B + S) induced a marked suppression of the tumour growth. The difference between group A + S or B + S and the control was significant.

The percentage of mammary tumours with different growth rates on day 16 is illustrated in Fig. 3. In all experimental groups, especially groups A + S and B + S treated with Sasa Health®, the percentage of tumours with growth rates higher than 200% was less than the control value. Conversely, group A + S or B + S had values higher than the control as well as group A or B in the percentage of tumours with growth rates lower than 100%.
3. Spontaneous motor activity

Fig. 4 shows the spontaneous motor activity. At 6 months of age, group A kept on the FIR rack tended to have more activity at total and light phases than group B kept on the normal rack. The differences became more marked at 10 months of age.

Fig. 3. Percentage of the number of mammary tumours with different growth rates on day 16 in each group. ■: <100%, □: 100-200%, ○: >200%. Number of tumours examined is in the parentheses.
4. Life span

As shown in Fig. 5, the life span considering the survival rate at each month between 4 and 12 months of age was higher for groups A and B than the control. Furthermore, it was higher for group A than group B between 7 and 12 months of age.

5. Growth of normal and preneoplastic mammary glands

Table I shows the growth of normal and preneoplastic mammary gland. The numbers of HAN in groups B and B + S were significantly lower than the control. In other parameters, little difference was observed between the control and either experimental group.

6. Weights of carcass and endocrine organs

Carcass weight at autopsy was slightly lower in groups A (29.5 ± 0.3), A + S (29.2 ± 0.5), B (29.1 ± 0.7) and B + S (27.2 ± 0.8) than the control (30.8 ± 0.6). Little difference was observed between groups in the weights of organs examined (data not shown).
Discussion

The present study shows that FIR irradiation between 1 month and 5 months of age apparently suppressed the formation of precancerous HAN; however, it had little effect on mammary tumour development. On the other hand, FIR irradiation after 5 months inhibited mammary tumour development, but little affected HAN formation. While mammary tumour growth was retarded to some extent by both FIR treatments in this study, the inhibition was much more marked when the treatment was given after the appearance of tumour\(^{15}\); the mean level of tumour-associated EGFR mRNA, which is essential for production of cell surface receptors that bind TGF\(\alpha\) and mediate its biological effects, was significantly decreased and anterior pituitary weight and serum leptin level were similarly declined by that treatment. Accordingly, growth inhibition may result from resistance of tumour cells to stimulatory autocrine and paracrine effects of factors such as TGF\(\alpha\), possibly by down-regulation of corresponding cell surface receptors. A decrease in anterior pituitary size may limit the synthesis and/or release of polypeptide hormones that directly or indirectly regulate tumour cell proliferation\(^{15}\). Thus, there would be a critical period for FIR to inhibit the development and/or growth of precancerous HAN and mammary tumours in mice. Incidentally, HAN and mammary tumour began to appear at 1 month and 5 months of age, respectively, in this strain\(^{19}\).

The partial inhibition of mammary tumour growth by FIR irradiation between 1 month and 5 months or after 5 months of age was enhanced by the combined treatment with extract of bamboo grass leaves (Sasa Health\(®\)). The results support the merit of using Sasa Health\(®\) as a complementary agent for hyperthermia; it stimulated the partial inhibition by FIR of tumour growth as usually observed in natural products, which sometimes encourage the agents having only moderate or slight effects, while not those with marked effects\(^{20}-23\).

Although most animals in all three groups eventually developed mammary tumours, the life span was elongated in the experimental groups receiving FIR irradiation compared to the control. This would principally be ascribed to the retarded mammary tumour growth in the experimental groups. Furthermore, the longer life span in the experimental mice treated with FIR after 5 months of age would be due to the greater inhibition of mammary tumourigenesis. The stimulated motor activity at an advanced age may contribute to the elongation of life span in FIR irradiated experimental animals. Life span was also elongated in mice continuously exposed to FIR after birth\(^{6}\). Finally, the findings in this and previous studies\(^{6}\) suggest that an appropriate application of FIR is beneficial at an advanced age.

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References


遠赤外線照射によるマウス乳癌発生の抑制および乳癌増殖におけるクマザサ抽出液併用の影響

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要 旨：5ヶ月齢の遠赤外線照射は自然発生乳癌高発系SHNマウスの乳癌発生を有意に抑制したが、1～5ヶ月齢の照射では抑制効果は見られなかった。一方、乳腺の前癌症状を1～5ヶ月齢に遠赤外線照射を行った場合のみ抑制された。このことは、乳腺の前癌症状あるいは乳癌の抑制には遠赤外線の照射時期が重要であることを示す。この両期間の遠赤外線照射によって乳癌の増殖は若干抑制されたが、抗腫瘍作用をもつクマザサ抽出液（ササヘルスイ）を飲水として併用することにより増殖抑制効果は顕著に促進された。また遠赤外線照射により10ヶ月齢の自発行動量は上昇し、生存率は延長した。以上の結果、適切な遠赤外線照射は癌の予防、運動量上昇などを介して健康に有益であることが示唆された。