Anti-carcinogenic activity of simvastatin during the promotion phase of radiation-induced mammary tumorigenesis of rats

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Pregnant Wistar-MS rats received whole body irradiation with 2.6 Gy γ-rays from a 60Co source at day 20 of pregnancy. Control rats were fed a basal diet and were implanted with a diethylstilbestrol (DES) pellet at 30 days after weaning. In the experimental group, rats were fed a diet containing simvastatin immediately after weaning and received a DES pellet at 30 days after weaning. A high incidence of total mammary tumours was observed in the rats fed the control diet and treated with DES for 1 year. The administration of dietary simvastatin together with DES treatment significantly decreased the incidence of mammary tumours. The development of adenocarcinoma in the control rats was significantly higher than that in the rats fed the simvastatin diet. After the administration of simvastatin to the experimental group for 1 year, the serum estradiol-17β concentration in these rats was markedly reduced, but that of prolactin was not. No significant difference was seen in the development of the mammary glands between rats fed the control diet and those fed the simvastatin diet by whole mount observations. Simvastatin feeding produced an increased development of ER+ PgR+ tumours and a reduced incidence of ER+ PgR+ tumours. These findings suggest that simvastatin has a potent preventive activity during the DES-dependent promotion/progression phase of radiation-induced mammary tumorigenesis.

Materials and Methods

Simvastatin was kindly supplied by Banyu Pharmaceutical Co. (Tokyo, Japan). [2,4,6,7-3H]Estradiol-17β (sp. act., 4 TBq/nmol) and [17α-methyl-3H]17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R5020) (sp. act., 3 TBq/nmol) were purchased from Du Pont/NEN Research Products (Boston, MA). Estra
diol-6-[(10-carboxymethyl)-oximino-(2-135)]iodohistamine) (sp. act., 74 TBq/nmol) was obtained from Amersham (Aylesbury, UK). DES and 3,3'-diaminobenzidine was obtained from Sigma (St Louis, MO). Pellets were prepared in a medical grade Silastic tube (Dow Corning Co., Midland, MI) and were filled with 3 mg of DES mixed with 27 mg of cholesterol. A basal diet (MB-1) of biscuit form was used for the control experiments. Diet containing 0.03% (w/w) simvastatin was prepared in the same form by Funabashi Farm (Chiba, Japan) and was sterilized by γ-rays (10 kGy). The major components of MB-1 are as follows: total carbohydrate, 54.1%; protein, 24.6%; fat, 4.0%; fibres, 3.8%; moisture, 7.7%; ash, 5.8%; their concentrations did not change by sterilization.

Introduction

Simvastatin (Figure 1), a synthetic derivative of lovastatin isolated from the culture filtrate of Aspergillus terreus (1), is a prodrug of a specific inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA*) reductase (2). This compound is lactone. The dihydroxy acid form converted from simvastatin inhibits markedly HMG-CoA reductase (2). Since HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis, simvastatin has been used as an antihyperlipidemic drug. Mevalonate, produced by HMG-CoA reductase, is also the precursor of isoprenoid formation. Transfer of a farnesyl group from farnesyl pyrophosphate to p21ras proteins is a universal post-translation modification among the cellular ras proteins.

Inhibition of HMG-CoA reductase by lovastatin leads to the blocking of p21ras farnesylation, thereby reducing its membrane localization (3,4). Simvastatin prevented DNA replication and cell proliferation by inducing a G1 block of the cell cycle of the human breast cancer cell line MCF-7 (5) which activated ras oncogene expression (6). For these reasons, HMG-CoA reductase inhibitors have received increasing attention as pharmacological tools for controlling tumour cell growth. Previous studies in our laboratory demonstrated that a significantly higher incidence of mammary tumours in the presence of the tumour promoter diethylstilbestrol (DES) is observed in rats irradiated during pregnancy (7,8). The present study was designed to evaluate the anti-carcinogenic activity of simvastatin against DES-dependent promotion/progression of radiation-induced mammary tumorigenesis in rats. The relationship between the chemoprevention of mammary tumorigenesis by simvastatin and the pharmacological activities of this agent are discussed.

Materials

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Animals and treatment

The rats used in the present study were treated and handled according to the Recommendations for Handling of Laboratory Animals for Biomedical Research compiled by the Committee of the Safety and Handling Regulations for Laboratory Animal Experiments in our Institute. Wistar-MS rats, bred in this Institute, were kept in a controlled environment (14 h light–10 h dark) at 23 ± 1°C. They received food and water ad libitum. Seventy-nine pregnant rats received whole-body irradiation with 2.6 Gy γ-rays (0.17 Gy/min) from a 60Co source at day 20 of pregnancy (the presence of a vaginal plug denoting of the 57 pregnant control rats (which were fed the basal diet, and hormone concentrations, and by Mann–Whitney t-test for the level of significance of Kd values for the receptors were determined by a radioligand binding assay. Statistical analyses were conducted by Student’s t-test for the level of significance of differences between pairs of mean values for body weight, organ weight and hormone concentrations, and by Mann–Whitney U-test for Kd and concentrations of the steroid receptors. All statistical analyses were performed using StatView-J4.5 software (Abacus Concepts Inc., Berkeley, CA). Probability values <5% were considered significant.

Results

Chemoprevention by dietary simvastatin on the development of mammary tumours

Of the 57 pregnant control rats (which were fed the basal diet, administered whole body irradiation with 2.6 Gy γ-rays at day 20 of pregnancy and then treated with DES after nursing), 50 (88%) rats developed mammary tumours during the experimental period of 1 year (Table I). The administration of dietary 0.03% simvastatin together with DES implantation in the 22 experimental rats (also irradiated in late pregnancy) significantly decreased the incidence (36%) of total mammary tumours (P<0.0001). There were no significant differences between the two groups in the number of mammary tumours per tumour-bearing rat or in the average latency period until appearance of the first mammary tumour. The Iball’s index for overall tumour development in the simvastatin-fed rats was 42% of that in the control rats. From histological examination, adenocarcinoma was seen in 29 (34%) of the 85 tumours examined in the control group, but in only two (13%) of the 15 tumours obtained from the simvastatin-fed group (P<0.009).

Biological effects of long-term administration of simvastatin

The final body and organ weights are summarized in Table II. No change of body weight was observed after administration of simvastatin for 1 year. The simvastatin feeding of DES-treated rats caused hypertrophy of the liver (P<0.05) and ovaries (P<0.05), and atrophy of the mammary glands (P<0.05) was observed. Pituitary weight was increased slightly by the administration of simvastatin, but no significant difference was observed. A few pituitary tumours (<20 mg) were obtained in the control rats, but no tumour in organs other than the mammary glands was obtained in the rats of the simvastatin-treated group.

Serum concentration of hormones and lipids

At the end of the experiment, the serum concentrations of ovarian and pituitary hormones were measured 1 year after the start of the administration of dietary simvastatin together with DES implantation in the irradiated rats (Figure 2). The serum estradiol-17β concentration in the rats fed the simvastatin diet was markedly reduced, to 20% of that observed in rats fed the control diet (P<0.05). No significant differences in progesterone (P = 0.086), prolactin (P = 0.903), LH (P = 0.064), FSH (P = 0.776) or TSH (P = 0.059) were observed between the two groups. In addition, simvastatin did not show any effect on the concentrations of triglyceride (P = 0.408), free cholesterol (P = 0.630) or cholesterol ester (P = 0.515) (Figure 3).

Immunohistochemistry of prolactin in pituitary glands

Although simvastatin did not reduce the elevated level of circulating prolactin in rats implanted with DES, the expression of prolactin in the pituitary glands of simvastatin-fed rats was compared immunohistochemically with that in the control rats. No significant difference in prolactin cell number or in staining intensity for prolactin was observed between the two groups (data not shown).

Effect of simvastatin on development of mammary glands

Whole mounts of inguinal mammary glands were examined to examine the effects of development and differentiation of the
Table I. Chemoprevention by simvastatin on the development of mammary tumours

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of rats used</th>
<th>Rat with tumours No. (%)</th>
<th>No. of tumours</th>
<th>Latency period (months)</th>
<th>Iball index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FA (%)</td>
<td>AC (%)</td>
<td>Per tumour bearing rat</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>57</td>
<td>50 (88)</td>
<td>56 (66)</td>
<td>1.9 ± 0.2</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>22</td>
<td>8 (36)</td>
<td>13 (87)</td>
<td>2 (13)</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.009</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aFibroadenoma.  
*bAdenocarcinoma.

Table II. Biological effects of long-term administration of simvastatin

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Simvastatin (n = 11)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>231.5 ± 3.3</td>
<td>234.9 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>10.0 ± 0.4</td>
<td>11.5 ± 0.6</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Uterus (g)</td>
<td>0.58 ± 0.04</td>
<td>0.86 ± 0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Ovaries (mg)</td>
<td>52.5 ± 3.9</td>
<td>64.0 ± 3.4</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Adrenals (mg)</td>
<td>90.8 ± 5.1</td>
<td>95.6 ± 6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Pituitary glands (mg)</td>
<td>15.2 ± 1.6</td>
<td>17.2 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mammary glands (g)</td>
<td>2.8 ± 0.2</td>
<td>1.5 ± 0.4</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of simvastatin treatment on ovarian and pituitary hormones in rat serum. a Concentration (pg/ml); b Concentration (ng/ml); *p<0.05. E2: estradiol-17β, LH: luteinizing hormone, FSH: follicle stimulating hormone, TSH: thyroid stimulating hormone, Prog: progesterone, PRL: prolactin.

Glands by simvastatin. In the irradiated rats fed the control diet, the mammary glands showed small alveolar buds due to the DES implantation (Figure 4). Alveolar buds with lactiferous ducts were also observed throughout the mammary glands of the irradiated rats treated with DES together with simvastatin for 1 year. In the morphological examination, the administration of simvastatin was observed to have caused no significant effect on the mammary glands developed by DES implantation.

ER and PgR in mammary tumours

Many (75%) of the mammary tumours which developed in the rats fed the control diet were of the ER+ PgR+ type, and only one tumour (2.5%) was ER– PgR– (Table III). Simvastatin-fed rats showed significantly increased incidence (31%, P<0.05) of ER– PgR– tumours and significantly decreased incidence (39%, P<0.05) of ER+ PgR+ tumours. Table IV shows the receptor concentrations and Kd values obtained when the ER and PgR in the cytosol fraction were analysed with a Scatchard plot. The maximum binding sites for ER and PgR in the fibroadenomas developed in the simvastatin-fed rats were significantly lower than those in the control. Also,
The enhanced expression or mutational activation of ras oncogenes has been detected in mammary tumours of mice (18), rats (19) and humans (20). The ras oncogene products, p21\textsuperscript{ras} proteins, are bound to the cell membrane after translational farnesylation (21). HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate, a precursor for the biosynthesis of cholesterol and farnesyl pyrophosphate, and this reductase is the rate-limiting enzyme in the mevalonate pathway in animal cells (22). Since simvastatin is a specific inhibitor of hepatic HMG-CoA reductase (2), decreased activity of this enzyme reduces the amount of farnesyl pyrophosphate available for the farnesylation of p21\textsuperscript{ras}. Ura et al. (23) have reported the cytotoxic effect of simvastatin on human pancreatic adenocarcinoma cells that have the mutant Ki-ras oncogene at codon 12. The proliferation of human mammary epithelial cells and of the breast cancer cell line MDA-231 was drastically reduced in the presence of an inhibitor of the HMG-CoA reductase (24). Thus, mevalonate seems to be of critical importance for the growth of both normal and cancer mammary cells. However, the serum concentrations of free cholesterol and cholesterol ester were not reduced by long-term administration of simvastatin in our present study. The activity of HMG-CoA reductase in rat liver was markedly inhibited by a single administration of simvastatin (2), but was elevated when rats were fed a diet containing the inhibitor for several days (25). The induction of HMG-CoA reductase activity by the continuous administration of simvastatin was the result of a dramatic increase in the reductase mRNA levels (26) and an enhancement of enzyme stability (27) in rats. Therefore, it is unlikely that one of the mechanisms in the chemoprevention of mammary tumours by long-term treatment with simvastatin is to suppress the farnesylation of p21\textsuperscript{ras} proteins.

Addo et al. (5) have reported that the growth of ER\textsuperscript{+} mammary tumour cells was inhibited by simvastatin. In our present study, the maximum binding sites for ER and PgR in both adenocarcinoma and fibroadenoma were reduced by simvastatin feeding, therefore, it would be considered to have preventive activity on the growth of hormone-dependent tumour cells. The results of the receptor analysis revealed that simvastatin somehow modulated the hormone-dependency of the radiation-induced mammary tumours. The mechanism for the negative regulation on those receptors by simvastatin is still unclear. The inhibition of tumour cell growth by HMG-CoA reductase inhibitor did not show specificity for the cell transformation which is dependent upon farnesylated ras protein (28). Simvastatin was able to induce G1 arrest of the cell cycle of ER\textsuperscript{+} mammary tumour cells in the absence of oestrogen (29). Since, in the present study, simvastatin markedly reduced the serum concentration of estradiol-17\textbeta{}, an inhibition of the proliferation of ER\textsuperscript{+} tumour cells may have occurred in the present experiment.

In conclusion, the DES-dependent promotion/progression of radiation-induced mammary tumorigenesis was inhibited by simvastatin. The administration of dietary simvastatin retarded the development of mammary tumours of ER\textsuperscript{+} PgR\textsuperscript{+} type or the receptor concentrations were lower than those of tumours obtained in the rats fed the control diet.

Acknowledgements

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References


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