Lack of Promotion of Estrogen-dependent Mammary Gland Tumors \textit{in vivo} by an Isopropanolic \textit{Cimicifuga racemosa} Extract

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ABSTRACT

\textit{Cimicifuga racemosa} (CR) is widely used in the treatment of menopausal symptoms. Mechanistic studies suggest that unlike hormone-replacement therapy, CR does not stimulate estrogen-receptor positive breast cancer cells. To evaluate CR safety, we performed an \textit{in vivo} investigation of a clinically tested isopropanolic CR extract. Mammary tumors were induced in Sprague Dawley rats \((n = 75)\) by the application of 7,12-dimethylbenz[a]anthracene. Five to nine weeks later, the animals were ovariectomized, allowed to recover, and administered daily doses of CR extract \((0.714, 7.14, \text{or } 71.4 \text{ mg/kg body weight per day})\) or control substances \((\text{estrogen/positive control: } 450 \mu\text{g/kg/day mestranol; or CR vehicle/negative control})\). The animals were sacrificed 6 weeks later, and tumor number, size, plasma hormone levels, and the weight of estrogen-sensitive organs were analyzed. In contrast to mestranol treatment, CR treatment did not stimulate cancerous growth. There were no significant differences in tumor number or size between the CR groups and the vehicle control. Likewise, prolactin, follicle-stimulating hormone, and luteinizing hormone levels and organ weights and endometrial proliferation were unaffected. The lack of mammary tumor-stimulating effects of this extract is of great significance in establishing the safety of CR extracts for treatment of menopausal symptoms in women with a history of breast cancer in which hormone-replacement therapy is contraindicated.

INTRODUCTION

\textit{HRT} is a widely prescribed for the management of menopausal symptoms. However, the safety of estrogen replacement for women with endometrial, ovarian, and breast cancers has been questioned after reports that estrogen stimulates proliferation of estrogen-receptor positive tumors \((1)\). On the basis of this concern, it has been recommended that women with estrogen-sensitive cancers seek alternative methods for alleviating menopausal symptoms.

Phytoestrogens, including soy and red clover, are popular alternative treatment options available for relief of menopausal symptoms. On the basis of their mechanism of action, however, soy and red clover may have side effects similar to estrogen \((2)\), thus increasing the risk of tumor growth and estrogen-receptor positive cell proliferation \((3)\). As such, it is important to evaluate the estrogenic effects of alternative therapies.

CR, commonly known as black cohosh, black snakeroot, and rattlesnake root, has been indicated as an effective medicinal herb offering relief of menopausal symptoms such as hot flashes, sweating, mood swings, irritability, and sleeplessness \((4-10)\). Although CR has been proven to be effective for menopausal symptom relief, its mechanism of action is unclear.

\textit{In vitro} studies of isopropanolic CR extracts on estrogen-receptor positive breast cancer cell lines suggest that CR, unlike estrogen and soy, does not stimulate cell proliferation \((11-13)\). Likewise, clinical evaluations of menopausal women in which levels of gynecologically relevant hormones are measured support the lack of estrogenic effect of isopropanolic CR-extract formulations \((14-16)\). In contradiction to these data, some studies suggest that other CR formulations \((mehnolic or ethanolic extracts) have estrogen-like action \((17-21)\).

To further study the estrogenic activity of an isopropanolic CR extract \((Remifemin; \text{Schaper & Brümmer GmbH & Co KG, Salzgitter, Germany})\), which has been noted to be effective in relieving clinical symptoms of menopause \((16)\), we performed an \textit{in vivo} experiment in ovariectomized female rats simulating the estrogen-deprived environment of menopausal women. To investigate the effects of CR on estrogen-receptor positive mammary gland cells, mammary tumor growth was DMBA-induced in female rats. Using this \textit{in vivo} estrogen-receptor positive breast cancer model, we sought to test for estrogen-like activity of a commercially available isopropanolic CR extract \((Remifemin; \text{Schaper & Brümmer GmbH & Co KG, Salzgitter, Germany})\).

Animals, DMBA Application, Tumor Measurement, Ovariectomy and Treatment. Fifty days after birth, single intragastric doses \((20 \text{ mg})\) of DMBA \((\text{CAS no. } 57-97-6; \text{Batch } 202.85\text{H0296; product no. D } 3254; \text{SIGMA-Chemie})\) dissolved in sesame oil were administered to female Sprague Dawley rats \((\text{Mol:SPRD; Mollegaard Breeding, Ejby, Denmark; } n = 75)\). All of the animals were housed in identical environments \((\text{two females per } 800\text{-cm}^2 \text{polycarbonate cage})\). After DMBA application, the test animals were palpated weekly for mammary tumor development. Mammary tumors were measured in mm \((\text{length } \times \text{width})\) using a caliper. The two-dimensional tumor area was calculated as an ellipse. Identification of mammary tumors \(>5 \text{ mm in diameter (5-9 weeks after tumor induction)}\), the rats were bilaterally ovariectomized \((\text{under Ketamin/Xylazin anesthesia})\).

The animals were not randomized before ovariectomy because the development of a tumor could not be predicted; however, measures were

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\textsuperscript{2} The abbreviations used are: HRT, hormone-replacement therapy; CR, \textit{Cimicifuga racemosa}; DMBA, 7,12-dimethylbenz[a]anthracene.

MATERIALS AND METHODS

Investigational Products and Control Substances. This study was conducted in correspondence with Good Laboratory Practices. The local German animal care and oversight committee approved the animal experiments in this study. The CR-extract formulation and the control vehicle \((\text{CR control})\), which was identical in all respects to the CR extract except that it was devoid of the active substance, were manufactured according to a validated manufacturing process and supplied by \text{Schaper & Brümmer GmbH & Co KG}. Mestranol \((17 \alpha\text{-ethinylestradiol-3-methyl ether; CAS no. 72-33-3, an active estrogenic steroid administered p.o., was used as positive control (product no. E 5001; SIGMA-Chemie, Deisenhofen, Germany})\).

CR extract and CR control were suspended in fresh water at a concentration of 100 mg/ml immediately before the intragastric administration by gavage \((10 \text{ ml/kg body weight})\). The CR extract was prepared and administered daily at 1-fold, 10-fold, and 100-fold the human therapeutic dose \((0.714 \text{ mg/kg, 7.14 mg/kg, and 71.4 mg/kg, respectively})\). Mestranol was dissolved in sesame oil at a concentration of 225 \(\mu\text{g/ml} and prepared in an intragastric application volume of 2 ml/kg body weight.

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RESULTS

Animal Health and Mortality. There were no statistically significant differences between the experimental groups (V, I, II, III, M) with respect to tumor latency, number of tumor carriers, and the distribution of tumor sizes (ANOVA + Dunnett’s Test). Life expectancy, evaluated using the Kaplan-Meier survival analysis technique, did not statistically differ among the CR extract (I, II, III) and control groups (V, M), and the general health of the animals was satisfactory throughout the study. Of the 71 animals, 8 died or had to be euthanized prematurely because of their moribund state. In two of these cases (both in the mestranol group), premature euthanasia was motivated by ethical considerations because of large mammary tumors.

Body Weight. Mestranol treatment caused a significant reduction in weight gain within 1 week of the start of treatment (P ≤ 0.05) as compared with the control and CR-extract treated groups (Fig. 1). No significant weight differences were noted between the CR-extract (I, II, III) and CR-control (V) groups.

Mammary Tumor Growth. Mammary tumor growth was significantly reduced after ovariectomy and the subsequent loss of natural estrogen secretion (Fig. 2). Mestranol, which induced tumor regrowth, increased growth of the mammary tumors significantly larger than those of the three CR-extract or CR-control groups (P ≤ 0.05; ANOVA + Dunnett’s test).

The three CR-extract and CR-control groups did not show significant differences in mammary tumor size. In CR-treated groups, decreased growth of the mammary tumors was observed compared with those of the CR controls, suggesting a possible inhibitory effect. However, statistical analysis (Ryan-Einot-Gabriel-Welsch multiple test) failed to demonstrate statistical significance.

Clinical Chemistry (Prolactin, Luteinizing Hormone, and Follicle Stimulating Hormone). The three doses of CR extract did not alter prolactin, luteinizing hormone or follicle stimulating hormone levels as compared with the CR-control group (Fig. 3). By contrast, and as expected for p.o. estrogen treatment, mestranol induced a significant increase in prolactin levels (P ≤ 0.001) and a significant decrease in luteinizing hormone and follicle stimulating hormone levels (P ≤ 0.001) compared with the CR-control animals (Fig. 3).

Postmortem Findings. Postmortem evaluations did not reveal significant differences between the CR-extract and CR-control groups. An apparent difference was noted between the animals treated with mestranol and the animals of the other four groups (CR vehicle and

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![Fig. 1. Development of the body weight. Mestranol treatment inhibits weight gain, whereas CR extract does not significantly affect normal weight gain. Between weeks 17 and 21, there was a significant difference between the body weight of mestranol-treated animals and control animals (P ≤ 0.05).](image-url)
CR extract I, II, III) with respect to total tumor count as well as malignancy (Table 1). Of the mestranol animals, 86% showed malignant tumor growth, compared with the 50% in the control group and 50%, 64%, and 47% in the three CR-extract groups (I, II, III), respectively. The number of malignant tumors per animal was significantly higher in the mestranol group compared with the other four groups ($P < 0.01$; Mann-Whitney, exact test, two-sided). Unlike the CR-treated and control animals, 50% ($n = 7$) of the mestranol-treated animals presented with four or more mammary tumors (Table 1). There were no differences between the CR-treatment and the control animals with respect to tumor number or malignancy. There was, however, a slight tendency toward reduced tumor growth in CR-treated rats.

A histopathological examination of the ovarian region, which confirmed a complete bilateral ovariectomy in all animals, revealed foreign body granulomas and low-grade round cell infiltrates in the ovarian region. Such findings are likely to be reactions to the suture material used after ovariectomy. The bilateral ovariectomies also resulted in atrophic uteri in the CR-vehicle control and CR-extract groups, an observation that is typical in estrogen-deficient animals. Conversely, the mestranol-treated group presented signs of hyperestrogenism, with hypertrophic uteri showing

Table 1  

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of tumors per animal</th>
<th>Total no. of malignant tumors, M</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR control</td>
<td>7 4 2 0 1 0 0 0 0</td>
<td>12</td>
</tr>
<tr>
<td>CR treatment</td>
<td>7.14 mg/kg</td>
<td></td>
</tr>
<tr>
<td>0.714 mg/kg</td>
<td>7 4 2 0 0 1 0 0 0</td>
<td>13</td>
</tr>
<tr>
<td>7.14 mg/kg</td>
<td>6 3 4 0 0 0 0 0 0</td>
<td>11</td>
</tr>
<tr>
<td>Mestranol</td>
<td>2 3 0 2 2 0 1 2</td>
<td>50</td>
</tr>
</tbody>
</table>
an increased incidence of endometrial squamous cell metaplasia and glandular-cystic hyperplasia.

The CR-extract and CR-control animals presented with a decreased uterine size, whereas the mestranol-treated animals had significantly increased absolute and relative uterus weight in comparison with the CR-treated and control animals. Furthermore, the CR extract studied does not promote further development of malignant mammary tumors as has been shown consistently for estrogen. Further research, however, is needed to determine the effect of CR extracts before the development of mammary tumors.

In summary, the treatment of ovariectomized Sprague Dawley rats with CR extract yielded no evidence of estrogenic effects on estrogen-sensitive hormone levels, the endometrium, or the growth of DMBA-induced mammary tumors. These data, in conjunction with the previously reported in vitro, in vivo, and clinical trial data, support the safe use of CR in estrogen-sensitive patients for which HRT is contraindicated.

REFERENCES


