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Concomitant administration of an isopropanolic extract of black cohosh and tamoxifen in the in vivo tumor model of implanted RUCA-I rat endometrial adenocarcinoma cells

Thomas Nißlein*, Johannes Freudenstein

Schaper & Brümmer Co., R & D-Department of Veterinary Medicine, Bahnhofstr. 35, 38259 Salzgitter, Germany

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Abstract

Black cohosh is a well known herbal remedy of long traditional use against menopausal complaints. Recently published studies on postmenopausal hormone replacement with synthetic substances associated severe negative side effects with an increase in duration of administration. The subsequent popularity of alternative treatments, often herbal drugs, made investigations into the safety of these preparations more pressing. Until now, black cohosh demonstrated no estrogen-agonistic activity in mammary cells, neither in animal model nor in cell culture, i.e., no gene transcription or cell proliferation was induced. Here we tested for the influence of a standardized isopropanolic extract of black cohosh on an animal model of endometrial cancer. Ectopic growth of the primary tumor as well as the incidence and localization of metastases were examined, partly in the setting of a combination treatment with tamoxifen. In contrast to the endometrial estrogen agonist tamoxifen, black cohosh did not further growth or metastasizing potential of the primary tumor. Absence of detectable supportive or antagonistic effects between both treatments most probable come from the relatively high tamoxifen dose.

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1. Introduction

Actaea (= *Cimicifuga*) *racemosa* (black cohosh) is a well known native American herbal remedy with an established record in European herbal medicine for the treatment of gynecological disorders such as irritation and congestion of the uterus, vagina, and cervix, as well as for promoting uncomplicated births, uterine

involution, and recovery. Recent clinical research with a standardized isopropanolic extract of the rhizome of black cohosh (iCR) suggests that its long proven clinical efficacy for alleviating menopausal symptoms such as hot flashes and night sweats is devoid of any accompanying systemic estrogen-agonistic activity (Liske et al., 2002), but might be explained by organ specific, selective estrogen receptor modulation (Riggs and Hartmann, 2003).

Well documented in vitro and animal studies showed absence of neoplasia induction or stimulation of proliferation in mammary tissue (Zierau et al.,

* Corresponding author.

E-mail address: thomas.nisslein@schaper-bruemmer.de (T. Nißlein).

2002; Bodinet and Freudenstein, 2002; Freudenstein et al., 2002). In order to provide additional safety data, we now performed an experiment with transplantable endometrial adenocarcinoma cells. Inbred Dark Agouti (DA/Han) rats are syngeneic to the permanent cell line RUCA I which has extensively been characterized (Vollmer et al., 1995, 1995a; Vollmer and Schneider, 1996). Tumors grow at the site of implantation and tend to metastasize into regional lymph nodes and lungs. By treatment of the implanted animals with iCR, we tested for potential effects on onset and growth of the primary tumor as well as on kinetics and localisations of metastases. One group of experimental animals was treated with the mixed estrogen agonist/antagonist tamoxifen in combination with iCR to test for supportive or antagonistic interactions.

2. Materials and methods

2.1. Animals and cells

Four groups of 5–6 female ovariectomized DA/Han rats, weighing between 180 and 195 g were included in the study.

RUCA-I cells (1×10^6) that had been cultivated in RPMI 1640 with phenol red and 10% fetal calf serum were subcutaneously administered to all experimental animals. The cells were deposited on the back of the animals just beside the lumbar part of the vertebral column.

One group of animals received iCR alone whereas a second group received the extraction medium of iCR, isopropyl alcohol in the corresponding dose. Two additional groups were treated with tamoxifen, an estrogen receptor ligand with uterine activity either alone or in combination with iCR.

2.2. Study drugs and administration

The iCR liquid extract was administered via the animals' drinking water in a dose that has previously been established to guarantee an effective uptake of 60 mg herbal drug/kg body weight. The mixture was freshly prepared on a daily base. Tamoxifen was given by subcutaneous injection at a daily dosage of 200 μ g tamoxifen/kg body weight. Treatments were started at the day of cell injection.

All conditions of husbandry were in accordance with local regulations, and experimental procedures were approved and conducted under the auspices of a local German animal care and oversight committee.

2.3. Clinical evaluation and necropsy findings

All animals were weighed and inspected twice a week, including palpation of the injection site. Palpatory inspection of axillary and inguinal lymph nodes was performed likewise.

After 5 weeks of tumor growth the animals were sacrificed by CO₂-asphyxiation and exsanguination, individual animals that had shown signs of distress during the course of the experiment were killed prematurely. Post-mortem examinations were done and all organs were checked macroscopically for abnormalities. Tumors, lungs, and lymph nodes were excised and evaluated microscopically.

3. Results

From day 3 after cell implantation all animals irrespective of treatment developed tumors at the site of the initial cell depot. Ectopic tumor masses grew rapidly towards the intestinal cavity, thereby infiltrating various adjacent tissues.

In the course of tumor development, body weight of untreated and iCR treated animals was relatively stable, whereas tamoxifen-treated animals lost approximately 5% and animals treated with a combination of tamoxifen and iCR lost even 10% of their initial body weight within 5 weeks (Fig. 1).

The mean tumor mass in the untreated control group after 5 weeks of growth was 570 mg (320–730 mg), whereas mean tumor mass after 5 weeks of tamoxifen-treatment was 660 mg (470–920 mg). Animals that were treated with iCR only had a mean tumor mass of 550 mg (180–860 mg), whereas the tamoxifen–iCR combination resulted in a mean tumor mass of 590 mg (360–1030 mg).

Relative tumor masses (% of controls) are given in Fig. 2.

Pulmonary metastases were frequently found in all groups. In the untreated and in the tamoxifen-treated groups >50% of the animals (3, resp. 4) showed pulmonary metastases, additionally two abdominal

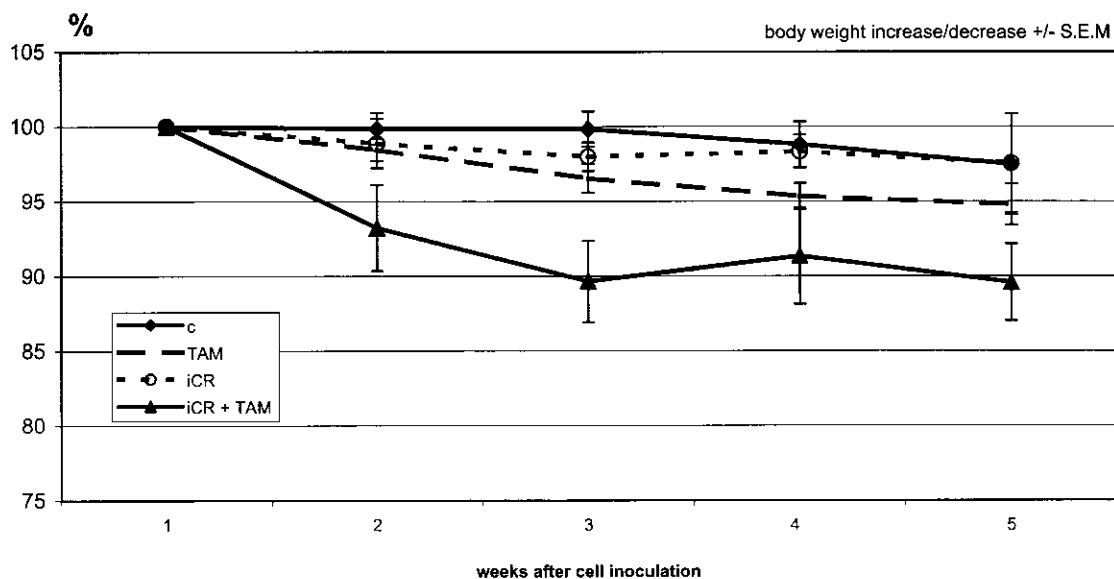


Fig. 1. Mean body weight development. Development of body weight, beginning at the time of cell inoculation. Mean alterations and standard errors of the mean (S.E.M.) are given for each treatment group. c = untreated controls, TAM = tamoxifen-treated animals, iCR = animals treated with black cohosh, iCR + TAM = animals treated with black cohosh and tamoxifen.

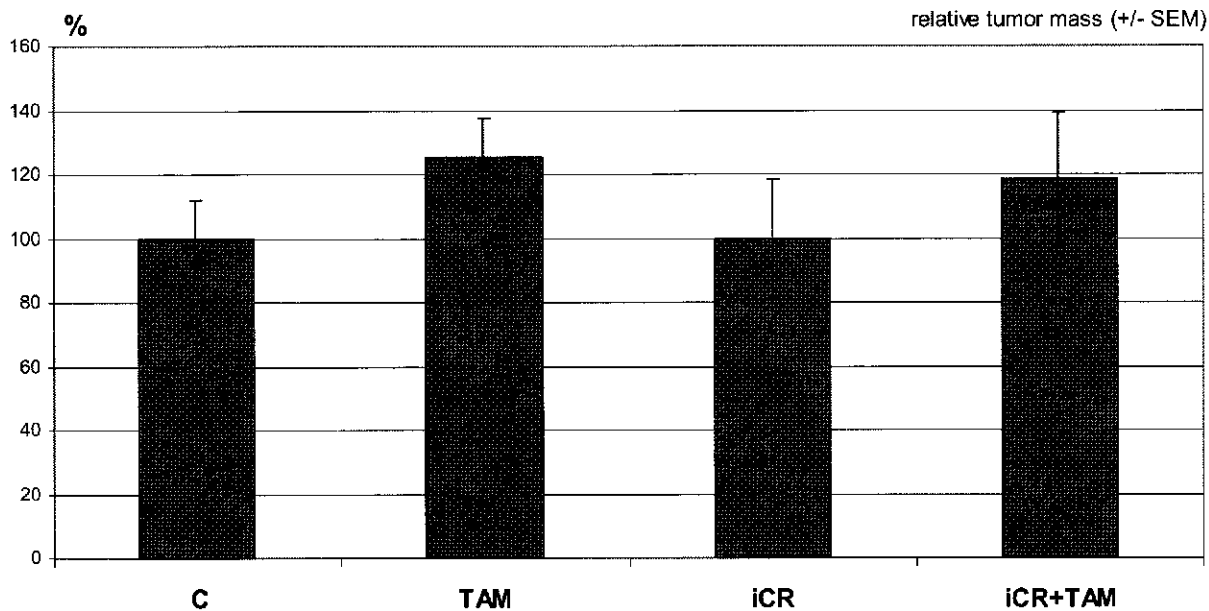


Fig. 2. Mean relative tumor mass. Necropsy findings after 5 weeks of tumor growth. Relative tumor weights (corrected for body weight, expressed as percentage of controls) and standard errors of the mean (S.E.M.) are given for each treatment group. c = untreated controls, TAM = tamoxifen-treated animals, iCR = animals treated with black cohosh, iCR + TAM = animals treated with black cohosh and tamoxifen.

Table 1
Metastasizing profile under experimental treatments

Group	Lung metastases (n)	Abdominal metastases (n)
Control	3/6	0/6
Tamoxifen	4/5	2/5
iCR	2/6	0/6
Tamoxifen + iCR	1/6	0/6

Necropsy findings after 5 weeks of tumor growth. Lung metastases and abdominal metastases as detected in gross pathology are given. n: number of metastasis bearing animals/number of total animals within each group.

metastases were found in tamoxifen-treated animals. Two animals produced metastases in the iCR-group, whereas only one of the TAM + iCR-treated animals developed metastases (Table 1).

4. Discussion

Animal and human data have demonstrated since long that in the uterus tamoxifen behaves like an estrogen-agonist (Ismail, 1999). The increase in RUCA cell-induced tumor mass under tamoxifen treatment as here observed is therefore in accordance with these findings (Taponeco et al., 2002). In contrast to this, in mammary tissues tamoxifen behaves like an estrogen antagonist, preventing, e.g., dimethylbenz(a)-anthracene induced mammary carcinoma (Jordan, 1976). In this model of chemically induced hormone-sensitive mammary tumor, iCR behaves like tamoxifen, in further increasing the number of tumor-free animals, prolonging the mean survival time of the experimental animals and in reducing the individual tumor burden (Niblein and Freudenstein, 2003).

In mammary tissue, no antagonism between iCR and tamoxifen has so far been observed even when iCR was administered under highly competitive surroundings, i.e., added on a sub-optimal tamoxifen dosing regimen (Niblein and Freudenstein, 2003).

Now we show that in uterus-derived tissue iCR does not augment the tumor promoting effects of tamoxifen, nor does it antagonize experimentally applied high doses of tamoxifen.

The slightly decreased metastasis rate in iCR-treated animals represents only an exploratory find-

ing, but shows however that iCR-treatment of rats carrying a highly metastasizing hormone-responsive neoplasia does not promote metastasis formation. Even though the precise mechanisms of metastasis formation have so far not been investigated, it has been shown that the heterodimeric glycoprotein clusterin is regulated by estrogens and antiestrogens in the RUCA-I/DA/Han-model. Clusterin is known to inhibit complement-mediated cytolysis, and therefore may protect the RUCA-I cells from immune surveillance, thus contributing to their highly metastasizing phenotype after implantation (Wünsche et al., 1998). Furthermore, matrix metalloproteinases MMP-2 and MMP-13, enzymes contributing to tumor invasion, migration and metastasis, are also regulated by estrogens and antiestrogens in the RUCA-I/DA/Han-model (Tüshaus et al., 2003).

Tamoxifen promotes the growth of the primary tumor, but also tends to aggravate its metastasizing potential. As only the second feature seems to be attenuated by a concomitant iCR-treatment, the putative effector mechanisms should differ from those that sustain the primary tumor. Matrix metalloproteinases are differently regulated by tamoxifen in either primary tumor or metastases (Tüshaus et al., 2003). Therefore they represent one possible pathway for iCR-mediated effects.

Future research should focus on characterizing animal cancer models where the primary tumor corresponds closely to human neoplasias. Any such model would additionally be substantiated, if parallel pathways of metastasis formation prevail.

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