Effects of Tamoxifen and Melatonin on Mammary Gland Cancer Induced by N-methyl-N-nitrosourea and by 7,12-dimethylbenz(a)anthracene, Respectively, in Female Sprague-Dawley Rats

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Abstract. Chemopreventive effects were analysed of antioestrogen TAM and of MEL on NMU- or DMBA-induced mammary gland cancer, respectively, in female Sprague-Dawley rats. NMU was administered intraperitoneally in two doses each of 50 mg/kg b.w. between 46th – 57th postnatal days. DMBA was given by gavage in one dose (20 mg per animal) between 50th – 54th postnatal days. The treatment with MEL began 12 days and the treatment with TAM 10 days before carcinogen administration; both chemopreventive substances were administered until the end of the experiment (24 weeks after carcinogen application). TAM was administered subcutaneously twice a week in a dose 2.5 mg/kg b.w. MEL was given in tap water (20 mg/ml) daily between 3 p. m. to 8 a. m. The tumour incidence, tumour frequency per group and animal, latency period, tumour volume, body weight gain in the rats and weight of uterus (in the experiment with NMU) were evaluated. TAM suppressed carcinogenesis to 0% incidence like TAM+MEL in both the NMU and DMBA models. In NMU-induced mammary carcinogenesis MEL lowered the tumour volume (although statistically non-significantly) by 30% in comparison with the control group; in DMBA-induced mammary carcinogenesis it lowered the tumour volume (2.70 ± 0.81 cm³ vs. 0.90 ± 0.33 cm³) and lengthened (non-significantly) the latency period (by 12 days). The weight gain of animals in both NMU and DMBA models and relative uterus weight in the NMU model were significantly lower in the groups treated with TAM and TAM+MEL as compared to the control group and the group treated with MEL. Evaluation of the combined effect of TAM+MEL was not possible due to total suppression of carcinogenesis by TAM.

TAM and TAM+MEL are highly effective agents in rat mammary carcinogenesis prevention, but the side effects of TAM in humans limits its use in clinical oncology.

Oestrogens have an important role in the process of breast cancer initiation (mitogenic activity). Binding to oestrogen receptors (ER) is the first active step in target tissue; antioestrogens competitively inhibit the binding of oestrogens to ER and through this action have an important role in the treatment of oestrogen-dependent breast tumours.

Experimental rat mammary carcinogenesis induced by chemocarcinogens is a widely used model for the study of oestrogen and antioestrogen role in the process of breast cancer initiation. N-methyl-N-nitrosourea (NMU) and 7,12-dimethylbenz(a)anthracene (DMBA) are the most frequently used chemical substances in mammary tumour induction; NMU is an alkylating substance acting directly as DNA adduct, whereas DMBA is a polycyclic aromatic hydrocarbon which damages DNA through epoxides rising from its metabolization (Russo and Russo, 1996).

The non-steroidal antioestrogen tamoxifen (TAM) is often used in the treatment of oestrogen-dependent breast tumours. Its efficacy was proved clinically (National Surgical Adjuvant Breast and Bowel Project-1; Fisher et al., 1998) as well as experimentally (Jordan, 1974). TAM acts as an antioestrogen in breast tissue whereas in other tissues it acts as a partial agonist. Oestrogen-like effects were observed in the increase of bone density, decrease of serum cholesterol and of low-density lipoproteins. On the other hand, TAM has a carcinogenic effect on the uterus (Fisher et al., 1994). Potential undesirable effects of TAM require modification of its application alone or in combination with other chemopreventive substances, which would enable the decrease of its dose and/or its side effects.

Melatonin (MEL), the main product of mammalian pineal gland, represents one of the ways to influence neoplastic growth in mammary tissue. Many *in vivo* and *in vitro* experimental studies reported tumour-suppressive effects of MEL by different mechanisms of action. Blask et al. (1991) point out the possible antioestrogen effects of MEL in NMU-induced rat mammary carcinogenesis. Tamarkin et al. (1981) supposed that the oncostatic effect of MEL in DMBA-induced mammary tumours in the rats might be caused by the suppression of plasma prolactin levels. Mollis et al. (1993) reported physiological concentrations of MEL to lower ER levels. Rato et al. (1999) explain the oncostatic activity of MEL by its efficacy to block the binding of oestradiol-ER complex to DNA. Cos et al. (1996a) found out that the antiproliferative effect of MEL on MCF-7 cells can arise from lowering DNA synthesis. In another paper MEL significantly lengthened the cell cycle of human tumour cell lines (Cos et al., 1996b), or stopped the cell cycle of MCF-7 cells (Mediavilla et al., 1999). MEL has also been suggested to have immunoenhancing properties and was shown to be a highly efficient scavenger of hydroxyl as well as peroxyl radicals (Reiter, 1995). The administration of MEL is not accompanied by side effects even in chronic experiments, and this fact together with the oncostatic properties cited above could complete its use in cancer prevention.

A presumption exists concerning the combination of several chemopreventive substances with different mechanism of efficacy to be more effective than application of an individual substance. The results of Wilson et al. (1992) point out an ability of MEL to potentiate the antiproliferative effect of TAM in MCF-7 human tumour cell lines. Kothari et al. (1997) reported high effectiveness of combined TAM and MEL administration in NMU-induced rat mammary carcinogenesis.

The aim of this work was to evaluate the efficacy of individual and combined TAM and MEL administration in chosen doses, in mammary carcinogenic process, in complex view of using both well-known carcinogens for the induction of mammary tumours – NMU and DMBA, respectively, in female Sprague-Dawley rats. The combined administration of TAM and MEL was evaluated mainly with regard to the different properties of the two substances. In addition, the results obtained with tamoxifen will serve as a starting point in our next experiment for comparison of the antineoplastic activity of raloxifene (antioestrogen used in the treatment of osteoporosis) in the mammary gland.

Material and Methods

Female Sprague-Dawley rats (AnLab Ltd., Prague, Czech Republic) aged 37–41 days were adapted to standard vivarium conditions (t = 23 ± 2°C), relative humidity 60–70%, artificial regimen light : dark – 12 : 12 (light on 7 a.m., 150 lux per cage). Animals were fed the MP diet (Top-Dovo, Dobrá Voda, Slovakia) and drank tap water ad libitum. Mammary carcinogenesis was induced by NMU (Sigma, Deisenhofen, Germany) administered intraperitoneally in two doses each per 50 mg/kg b.w. between 46th – 57th postnatal days (the second dose of NMU was given 7 days after the first one). NMU was freshly prepared and dissolved in physiological solution. DMBA (Sigma, Deisenhofen, Germany) was dissolved in corn oil and applied by gavage in one dose (20 mg per animal) on 50th – 54th postnatal day. Animals were divided into 4 groups: 1. control group (without chemoprevention), 2. chemoprevention with TAM, 3. chemoprevention with MEL, 4. combined chemoprevention with tamoxifen and melatonin (TAM+MEL). In the experiment with NMU each group consisted of 20 animals, in the experiment with DMBA each group was formed by 25 animals.

TAM (base, Lachema, Brno, Czech Republic) was administered twice a week subcutaneously in the dorsal interscapular area in a dose 2.5 mg/kg b.w. as 0.25 ml of corn oil (Sigma, Deisenhofen, Germany) solution. MEL (Biosynth, Staad, Switzerland) was administered as a solution in tap water in a concentration 20 µg/ml daily between 3 p.m. and 8 a.m. (from 8 a.m. to 3 p. m. animals were drinking tap water). For preparation of 1 l of solution, 20 mg of MEL were dissolved in 0.4 ml of 30% ethanol and mixed up with tap water to the desired volume. Chemoprevention with TAM began 10 days and with MEL 12 days before carcinogen administration. All animals were weighed and palpated weekly to register the presence, number, location and size of palpable tumours. During 12th and 13th weeks of the experiment we recorded the food intake of animals. On 24th week all rats were sacrificed by quick decapitation, mammary tumours and uteri were investigated, removed and weighed, the size of tumours was registered. The body weight gains were calculated from 40th postnatal day until the end of the experiment. The experiment with NMU was carried out from November to May and the experiment with DMBA was carried out from June to December. The tumour incidence was evaluated by Mann-Whitney U-test, tumour frequency and latency period by one-way analysis of variance, and for evaluation of tumour volume, body weight gain and relative uterine weight, Kruskal-Wallis test was used. The tumour volume was calculated according to: $V = \pi \cdot (S_1)^2 \cdot S_2/12$ (S_1 and S_2 are the tumour diameters, $S_1 < S_2$).

Results

The incidence, tumour frequency per group and animal, latency period, tumour volume, body weight gain and relative uterine weights at the end of experiments are outlined in Tables 1, 2 and 3. Total mammary tumour growth suppression was registered in the group treated with TAM and with the combination of TAM and MEL, both in the experiment with NMU and DMBA. In the DMBA experiment, the tumour volume