

## Short Communication

# Preventive Effect of Indomethacin and Melatonin on 7,12-dimethylbenz/a/anthracene-Induced Mammary Carcinogenesis in Female Sprague-Dawley Rats. A Preliminary Report.

( indomethacin / melatonin / breast cancer / rat )

K. MÔCIKOVÁ-KALICKÁ, B. BOJKOVÁ, E. ADÁMEKOVÁ, M. MNÍCHOVÁ-CHAMILOVÁ, P. KUBATKA, E. AHLERSOVÁ, I. AHLERS

Institute of Animal Physiology, Faculty of Science, P. J. Šafárik University, Košice, Slovakia

**Abstract.** The aim of the experiment was to analyse the oncostatic effect of nonsteroidal antiinflammatory drug INDO, hormone MEL and combination of both substances in DMBA-induced mammary carcinogenesis in female SD rats. Chemoprevention started 10 days before the application of the first dose of DMBA to 35-day-old rats. INDO was administered in tap water (20 µg/ml of water) for 3 days in a week (days 2, 4 and 6), MEL solution in the concentration of 20 µg/ml of tap water was administered between 3 p.m. and 8 a.m. for 4 days in a week (days 1, 3, 5 and 7); during other days the animals drank tap water only. In combined chemoprevention, rats were drinking solutions of INDO and MEL according to the above-mentioned scheme. DMBA in the dose of 10 mg/rat was administered intragastrically using a probe to all rats 3 times on postnatal days 45, 50 and 55. There were four experimental groups: group 1 – without chemoprevention, group 2 – INDO treatment, group 3 – MEL treatment, group 4 – application of INDO + MEL. The experiment lasted 26 weeks from the first administration of DMBA, when the final incidence and frequency of tumours per animal and group, as well as latency and average volume of tumours were evaluated. The con-

tent/concentration of malondialdehyde (MDA) was determined in selected tissues as a criterion of lipoperoxidation, considering its potential influencing by chemoprevention.

The tumour incidence in controls was 100%; INDO reduced the incidence (36.84%) and frequency per group and animal, decreased the mean volume of tumours and prolonged the latency. Chemoprevention using combination of INDO with MEL was successful like that with INDO; however, it did not influence the tumour volume. MEL decreased the incidence to 42.11% and pronouncedly reduced the tumour frequency per group. INDO, administered alone or in combination with MEL, reduced an increased content/concentration of MDA in the liver, bone marrow and serum of tumour-bearing rats. INDO, MEL and INDO + MEL had a pronounced chemopreventive effect and showed to be a favourable combination in prevention of experimental mammary carcinogenesis.

Three ways of influencing the cancer disease can be named: improved diagnostics with recording of early stages of changes, improvement of the treatment approach and prevention. In the future, the third way should be preferred. In women, the problem of breast cancer dominates and chemoprevention of this disease represents one of the key roles of experimental and clinical oncology. Along with standard and promising selective modulators of oestrogen receptors and retinoid groups, the non-steroidal antiinflammatory drugs (NSAIDs) are also hopeful for their using in chemoprevention of tumours. In the tumour tissue (e.g. in experimental carcinomas of the mammary gland in rats; Cohen and Karmali, 1984) high levels of prostaglandins, originating from the activation of the arachidonic acid cascade, have been found; NSAIDs inhibit their formation.

Received August 28, 2000. Accepted December 21, 2000.

The project 1/7397/20 was supported by VEGA, Ministry of Education, Slovakia. The experiments were conducted according to the principles of the Law No. 115/1995 § 24 of Slovakia for the Care and Use of Laboratory Animals.

Corresponding author: Eva Ahlersová, Institute of Animal Physiology, Faculty of Science, P. J. Šafárik University, Moyzesova 11, 043 67 Košice, Slovakia.

Abbreviations: DMBA – 7,12-dimethylbenz/a/anthracene, INDO – indomethacin, MDA – malondialdehyde, MEL – melatonin, PGs – prostaglandins, SD – Sprague-Dawley.

Indomethacin (INDO), as a potent inhibitor of two isoforms of a key enzyme of prostanoid formation, cyclooxygenase (COX), constitutive COX-1 and inducible COX-2, is employed in clinical practice, especially in the treatment of rheumatoid diseases. The oncostatic properties of NSAIDs and their representative INDO can be looked for in induction of apoptosis of tumour cells in the mammary gland and colorectal region (Fosslien, 2000), in the inhibition of immunosuppressive effects of some prostaglandins (Rita and Young, 1994), in antiangiogenic properties (Tsuji et al., 1998), in inhibition of carcinogenic epoxides from polycyclic aromatic hydrocarbons, in inhibition of DNA adduct formation, one of the end-products of the arachidonic acid cascade – malondialdehyde (MDA) (for review see Fosslien, 2000).

INDO inhibited *in vivo* 7,12-dimethylbenz/a/anthracene (DMBA)-induced mammary carcinogenesis in female Sprague-Dawley rats (SD) (McCormick et al., 1985). In *in vitro* experiments with using the human tumour cells of mammary gland MDA-MB-231, the oncostatic effect of INDO was predetermined by its dose and presence of linolenic acid in the culture (Noguchi et al., 1995).

In the pleiotropic effect of the pineal hormone melatonin (MEL), besides the antigonadotrophic effect, the capability to synchronise circadian and partially circannual rhythms, also the scavenger effect of free oxygen radicals has been found (Reiter, 1995) as well as the immunomodulating effect mediated through the opiate system and some cytokines (Maestroni et al., 1994). The oncostatic effect of MEL may be explained by the presented properties; however, the inhibition of the oestrogen and prolactin effects is considered as prominent (for review see Cos and Sánchez-Barceló, 2000). In the culture of human mammary cells MCF-7, MEL acted directly on the cell cycle by prolonging its duration, while effective appeared to be only "physiological" concentrations of the hormone ( $10^{-9}$ – $10^{-11}$  M); higher and lower doses were not effective (Hill and Blask, 1988). The effect of exogenous MEL is dependent on the time of the day; the most pronounced effect has been observed at the end of light and in darkness (Cos and Sánchez-Barceló, 2000).

The aim of our work was to verify the oncostatic effectiveness of INDO, MEL and combination of INDO plus MEL in DMBA-induced mammary carcinogenesis in a sensitive strain of female SD rats. We supposed that the effects of both substances could complement each other or enhance the effect of an individually acting chemopreventive substance. Analysis and comparison of the effect of INDO with nimesulide, a selective inhibitor of COX-2 activity, under the same conditions and in combination with MEL will be performed within the second stage of the project.

## Material and Methods

Thirty-three–thirty-seven days old female SD rats (AnLab Ltd., Prague, Czech Republic) were used. All animals were housed in the vivarium under standard conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity (60–70%) and light regimen (light : dark – 12 : 12 h, light on at 7 a.m. with an intensity of 150 lux in a cage). Rats were fed the MP diet (Top-Dovo, Dobra Voda, Slovakia) with fat content of minimally 2.5% and drank water *ad libitum*. Chemoprevention started immediately after housing and lasted until the end of the experiment, 26 weeks from the first administration of DMBA. Rats were drinking INDO (Léčiva, Prague, Czech Republic) in the concentration of 20 µg/ml of tap water 3 days a week daily (Tuesday, Thursday, Saturday); for preparation of 1000 ml of solution 20 mg of INDO were dissolved in 3.1 ml of 60% ethanol and mixed up with tap water to desired volume. MEL (Biosynth, Staad, Switzerland) in the concentration of 20 µg/ml of tap water was administered between 3 p.m. and 8 a.m. (during the rest of the day, rats were drinking tap water) 4 days a week (Monday, Wednesday, Friday, Sunday). The solution of MEL was freshly prepared 3 times a week (20 mg of MEL were dissolved in 0.4 ml of 30% ethanol and mixed up with tap water to the volume of 1000 ml). In combined chemoprevention, rats drank solutions of INDO and MEL according to the above-mentioned scheme. On postnatal days 45, 50 and 55, all animals were given DMBA (Sigma, Diesenhofen, Germany) dissolved in corn oil in the concentration of 10 µg/rat intragastrically. The animals were divided into four experimental groups:

1. DMBA – control group
2. DMBA + INDO
3. DMBA + MEL
4. DMBA + INDO + MEL

Rats were weekly weighed and palpated; the presence, localization, number and size of tumours were recorded. Between weeks 15 and 16 of the experiment the daily intake of food and water was measured in all rats in order to find out the mean daily consumption of both parameters and to calculate the amount of chemopreventive substances in tap water. Twenty-six weeks after administration of the first dose of DMBA (the experiment lasted from November to June) rats were quickly decapitated, tumours were excised, weighed, measured and fixed in formol for histological analysis. In the serum, bone marrow and liver, the concentration/content of malondialdehyde (MDA) (Satch, 1978) was determined as a criterion of lipoperoxidation. Statistical significance of the differences in the final incidence of tumours was evaluated by Mann-Whitney U test, and that of final latency, tumour frequency per animal and group, mean tumour volume, body weight, food and water intake were evaluated by Kruskal-Wallis test. For evaluation of concentration/content of MDA, the one-way analysis of variance was used.