Results

The incidence, latency period, frequency and volume of mammary tumours at the end of experiment and the body weight gain are outlined in Table 1.

The incidence of mammary tumours in the control group was 100%. INDO significantly decreased the incidence and frequency of tumours per group ($P < 0.001$), volume of tumours and their frequency per rat ($P < 0.05$) and prolonged the latency ($P < 0.01$). Chemoprevention with MEL significantly decreased the incidence and frequency of tumours per group ($P < 0.001$), but it did not influence the other parameters. Combination of INDO with MEL decreased the incidence and frequency of tumours per group ($P < 0.001$) and animal ($P < 0.05$); prolonged the latency ($P < 0.05$) like INDO alone, but did not reduce the tumour volume. Weight gains were not significantly influenced by application of chemopreventive substances. The mean daily intake of food and water in rats did not differ statistically from that in controls (Table 2). The mean daily dose of INDO per rat was 0.64 mg, MEL 0.45 mg, in combination of chemopreventive substances it was 0.61 mg of INDO plus 0.52 mg of MEL. In comparison with intact animals, application of DMBA pronouncedly increased ($P < 0.05$) the content of MDA in the liver (260.09 ± 10.65 *vs* 174.26 ± 10.04 mmol) and its concentration in bone marrow (57.18 ± 3.38 *vs* 38.9 ± 4.00 mmol/g) in tumour-bearing rats. Application of INDO decreased the content of MDA (193.01 ± 22.17 mmol, $P < 0.05$) in the liver; combination of INDO plus MEL significantly reduced the concentration of MDA in bone marrow (30.20 ± 4.51 mg/g, $P < 0.05$). Changes in the concentration of MDA in the serum were insignificant after application of a carcinogen, INDO + MEL significantly decreased the MDA concentration compared to the group treated with MEL (3.19 ± 049 *vs* 4.49 ± 0.26 mmol/l, $P < 0.05$) in tumour-bearing rats.

Discussion

Indomethacin – a non-specific inhibitor of both isoforms of COX, a key enzyme of synthesis of prostanoids, successfully reduced the incidence of tumours by 63%, decreased their frequency per group and animal, decreased the mean volume of tumours and prolonged the latency. The dose of INDO used in our experiment – 20 μg/ml (10^{-8} M), alone or in combination with MEL, had a pronounced antioxidant effect; a decrease in the elevated content/concentration of malondialdehyde was recorded in the liver, bone marrow and serum of tumour-bearing rats. The side effects of INDO were not observed, the weight gains of animals in individual experimental groups were almost the same. The chemopreventive effect of INDO in the DMBA model of mammary carcinogenesis in female SD rats was also recorded by McCormick et al. (1985). They compared a short-term effect of INDO (25 mg and 50 mg/kg of diet), administered a week before and 2 weeks after application of DMBA, as well as its effect in chronic application (with the onset one week after DMBA administration) lasting 21 weeks. A pronounced reduction in tumorigenesis was recorded in both variants of the onset and duration of chemoprevention; above all, at a higher dose of INDO. Indomethacin inhibits cooxidation of polycyclic aromatic hydrocarbons (originating also at biotransformation of DMBA), which depends on the COX activity (Sivarajah et al., 1981). The activated COX leads to production of PGs, of which prostaglandin E_2 (PGE_2) specifically induces local biosynthesis of mitogenic acting oestrogens via the aromatase gene CYP-19 (Zhao et al., 1996). In the target tumour cells,

Table 1. The effect of INDO and MEL on DMBA-induced mammary tumour incidence, latency, frequency, average tumour volume and body weight gain in female SD rats

Experimental group	Number of animals	Tumour incidence %	Tumour latency /days/	Tumour frequency per group	Tumour frequency per rat	Average tumour volume /cm³/	Initial body weight /g/	Final body weight /g/	Body weight gain /g/
DMBA	15	100 %	93.40 ± 6.37	3.40 ± 0.63	3.40 ± 0.63	2.69 ± 0.66	121.70 ± 1.03	256.88 ± 10.42	135.18 ± 10.03
DMB+INDO	19	36.84 %♣	152.29 ±10.42♦	0.40 ± 0.13♣	1.14 ± 0.14 *	2.24 ± 1.45 *	128.95 ± 3.84	278.00 ± 3.13	149.05 ± 4.59
DMBA+MEL	19	42.11 %♣	100.25 ± 17.24	0.88 ± 0.34♣	1.88 ± 0.61	2.71 ± 0.88	125.10 ± 2.86	286.33 ± 10.54	161.23 ± 13.13
DMBA+INDO +MEL	19	31.57 %♣	137.50 ± 16.40 *	0.37 ± 0.11♣	1.00 ± 0.00 *	3.75 ± 1.39	134.00 ± 1.81	254.85 ± 6.61	120.85 ± 5.83

Data are expressed as means ± S.E.M.; significances of differences between control group and other groups are expressed as ♣ - $P < 0.001$, ♦ - $P < 0.01$, * - $P < 0.05$. DMBA - control group treated only with DMBA, DMBA + INDO - group treated with INDO, DMBA + MEL - group treated with MEL, DMBA + INDO + MEL - group treated with INDO and MEL

Table 2. The effect of indomethacin and melatonin on food and water intake in DMBA-induced carcinogenesis in female SD rats

Experimental group	Daily food intake per rat /g/	Daily water intake per rat /ml/
DMBA	19.66 ± 1.34	27.63 ± 1.06
INDO	17.03 ± 0.91	31.90 ± 2.23
MEL	21.40 ± 2.30	25.86 ± 0.68
INDO + MEL	21.66 ± 2.12	27.83 ± 1.12

Data are expressed as means ± S.E.M.

INDO increased the natural and antibody-dependent lymphocyte cytotoxicity (Droller et al., 1978) and inhibited the immunosuppressive effects of some PGs (Rita and Young, 1994), which probably contributed to facilitation of NK cell activity and stimulation of cytolytic lymphocytes (Golab et al., 2000). Angiogenesis accompanying the growth of experimental colorectal carcinomas in mice was successfully reduced by INDO (Marnett, 1992; Golab et al., 2000). By similar above-mentioned mechanisms, INDO inhibited the growth of transplanted mouse adenocarcinoma of the large intestine (Sato et al., 1983). Under *in vitro* conditions, INDO reversibly inhibited proliferation of cells of various breast tumour cultures (Bayer et al., 1979; Fulton, 1984). The dose-dependent effect of INDO was found out in the human cell line of the breast carcinoma 13762 MAT. INDO at the concentration of 10^{-4} M inhibited proliferation and markedly decreased production of PGE_2 and LTB_4 in tumour cells, which also testifies to the inhibition of lipooxygenase pathway in the arachidonic acid metabolism. Lower doses if INDO (10^{-6} – 10^{-10} M) stimulated proliferation of tumour cells (Tripathi et al., 1996). Very interesting is the effect of a low dose of INDO (0.005% w/w) administered in a diet with a high content of fat (20% corn oil), which pronouncedly reduced tumorigenesis but increased the volume of mammary carcinomas in the DMBA model in female SD rats (Noguchi et al., 1991).

In a series of our experiments investigating chemoprevention of experimental mammary carcinogenesis using melatonin alone or in combination with other substances, the presented work has brought the most convincing results. The incidence of mammary tumours at application of MEL was decreased by 58%; it exceeds a 30% drop after chemoprevention with MEL in N-methyl-N-nitrosourea-induced tumorigenesis, where retinyl acetate (Bojková et al., 2000) or tamoxifen (Kubatka et al., 2001) was used as the second chemopreventive substance. The incidence decreases in the works cited, however, were not significant. The decrease in the frequency of tumours per group at the unchanged latency period was significant in this experiment. MEL, administered alone in chemoprevention of mammary tumours, at some other time decreased only the volume of DMBA- and gamma irradiation-induced tumours (Môciková et al., 2000), or decreased only the frequency of tumours per animal in DMBA-induced mammary carcinogenesis (Ahlersová et al., 2000).

In the present experiment, combination of both chemopreventive substances (INDO + MEL) was also very successful; besides a significant decrease in the incidence and frequency of mammary tumours per animal and group it prolonged latency, too.

After comparison of more of our trials, we can confirm a higher effectiveness of lower daily doses of MEL administered discontinuously, at the end of light and during the dark part of the day. Higher supraphysiological doses of MEL in *in vitro* (and probably also in *in vivo*) experiments do not influence the activity of oestrogen receptors in the cells of breast carcinomas, whereby MEL loses its substantial oncostatic property (Rato et al., 1999).

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