Table 1. Chemopreventive effects of TAM and MEL in NMU-induced mammary carcinogenesis in female Sprague-Dawley rats

Group	CONT (n = 20)	TAM (n = 18)	MEL (n = 16)	TAM+MEL (n = 14)
tumour-bearing animals	16	0	9	0
number of tumours	44	0	32	0
tumour incidence (%)	80.0	0	56.3	0
tumour frequency per group*	2.20 ± 0.49	-	2.00 ± 0.72	-
tumour frequency per animal*	2.75 ± 0.53	-	3.56 ± 1.03	-
tumour latency* (days)	107.94 ± 6.67	-	109.00 ± 8.50	-
tumour volume* (cm^3)	1.39 ± 0.81	-	0.96 ± 0.30	-

*Data are expressed as means ± SEM.
CONT – control group

Table 2. Effects of TAM and MEL on body and uterine weights in the NMU-carcinoma model

Group	Body weight (g)		Body weight gain (g)	Uterine wet weight	
	Start of prevention	Necropsy		Absolute (g)	Relative* (%)
CONT	130.78 ± 2.03	251.56 ± 9.47	120.78 ± 9.51	0.647 ± 0.0552	0.246 ± 0.0204
TAM	128.00 ± 2.46	210.89 ± 3.60^{b,c}	82.89 ± 3.49^{a,b}	0.229 ± 0.0240^{a,b}	0.109 ± 0.0121^{a,b}
MEL	125.13 ± 2.17	263.13 ± 7.66	138.00 ± 7.16	0.685 ± 0.0520	0.271 ± 0.0196
TAM+MEL	127.11 ± 1.86	203.72 ± 4.69^{b,c}	76.61 ± 4.97^{a,b}	0.177 ± 0.0062^{a,b}	0.084 ± 0.0032^{a,b}

Data are expressed as means ± SEM.
Significantly different: $^aP < 0.0001$ vs. CONT, $^bP < 0.0001$ vs. MEL, $^cP < 0.0003$ vs. CONT
*Relative organ weight (%) = [absolute organ weight (g)/body weight (g)] × 100.

Table 3. Chemopreventive effects of TAM and MEL in DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats

Group	CONT (n = 24)	TAM (n = 25)	MEL (n = 25)	TAM+MEL (n = 24)
tumour-bearing animals	16	0	15	0
number of tumours	37	0	38	0
tumour incidence (%)	66.6	0	60.0	0
tumour frequency per group*	1.54 ± 0.36	-	1.52 ± 0.44	-
tumour frequency per animal*	2.31 ± 0.43	-	2.53 ± 0.60	-
tumour latency* (days)	100.13 ± 7.14	-	112.53 ± 7.96	-
tumour volume* (cm^3)	2.70 ± 0.81	-	0.90 ± 0.33^a	-
body weight gain* (g)	118.71 ± 5.32	93.52 ± 3.36^{b,c}	110.92 ± 3.60	98.67 ± 3.32^{d,e}

*Data are expressed as means ± SEM.
Significantly different: $^aP < 0.05$ vs. CONT, $^bP < 0.001$ vs. CONT, $^cP < 0.001$ vs. MEL, $^dP < 0.01$ vs. CONT, $^eP < 0.05$ vs. MEL

was decreased and the latency period was non-significantly lengthened (by 12 days) in the MEL group; when compared to the control group, the incidence and frequency was non-significantly decreased. In the NMU experiment the incidence in the control group was 80%, in the group with MEL the incidence was decreased by 30%, but this decrease was not statistically significant. No significant changes of the frequency, tumour volume and latency period were observed in groups treated with chemoprevention when compared to the control group in the NMU experiment. The investigation of mammary tumours confirmed a relatively stable relation between adenocarcinomas and fibroadenomas (rate about 70 : 30%).

The body weight gains in both experiments and relative uterine weights in the NMU experiment were significantly lower in the groups treated with TAM and TAM+MEL when compared to the group treated with MEL and the control group (Table 2). The decrease of body weight in TAM-treated rats at the end of experiment highly correlated with the decrease of absolute uterine weight ($r = 0.538$; $P < 0.0001$).

Discussion

The results of this study showed the potent inhibitory effect of TAM: chemically induced mammary carcinogenesis in groups treated with TAM and TAM+MEL was totally suppressed in both the NMU and DMBA experiments. The efficacy of TAM was also confirmed in other experimental studies where the mammary gland carcinomas were induced by NMU (Martin et al., 1996; Kothari et al., 1997) or by DMBA (Hollingstworth et al., 1998).

The effectiveness of TAM in mammary carcinogenesis prevention can be explained by several mechanisms. Principally, TAM acts as a competitive inhibitor of oestrogen binding to ER (Lippman et al., 1976). Fattman et al. (1998) revealed the ability of TAM to dephosphorylate the RB protein, resulting in the accumulation of cells in the pre-apoptotic G1 phase of cell cycle in MCF-7 and MDA-MB 231 cells. In TAM-treated MDA-MB 231 cells the increase of proapoptotic Bax protein, transforming growth factor (TGF-β) and transcription factor c-myc was observed, suggesting their possible participation in TAM-induced apoptosis (Fattman et al., 1998). Other possible anticarcinogenic effects of TAM may be the inhibition of Ca^{+}- calmodulin function (Lam, 1984) or inhibition of Ca^{+}-phospholipid-dependent protein-kinase activity (O'Brien et al., 1986), as well as the antioxidant activity of TAM (Wiseman and Quinn, 1994). Resulting from clinical observations, TAM was acknowledged as the standard substance in breast cancer treatment. However, long-term observations reveal its seldom but prominent side effects. In human breast tissue TAM acts as an antioestrogen, whereas in other target tissues of the organism it acts as a partial agonist. Numerous clinical studies point out the connection between endometrial pathology such as uterine abnormalities (Cohen et al., 1994), endometrial carcinomas (Fisher et al., 1994) and TAM treatment in postmenopausal women. Beside that, its carcinogenic effect on rat liver has been well documented (Hirsimaki et al., 1993).

In order to decrease the TAM toxicity but not its efficacy we considered its optimal dose. Thompson and Ronan (1986) achieved suppression of NMU-induced mammary carcinogenesis by more than 75% using the dose of 5 mg/kg of diet. In the case of 1 mg/kg in the diet the efficacy decreased to 35%. Doses of cca 200 μg/kg in the diet were ineffective (Moon et al., 1994). Martin et al. (1996) administered TAM daily in a dose 1 mg/kg b.w. The dose chosen by us – 2.5 mg/kg b.w. – administered twice a week showed to be very effective in tumour growth suppression. The body weight gain of animals receiving TAM in both carcinogen experiments was significantly decreased. This cannot be explained only by the absence of tumours in these groups, and we did not prove any suppression of appetite in these animals. The average food intake per animal was very similar in TAM and TAM+MEL groups when compared to groups without TAM administration – the control group and MEL (average values: 19.1 g/day vs. 19.2 g/day in 12^{th} and 13^{th} week of experiments). These results are in accordance with other experimental studies: the treatment with TAM led to a decrease in white adipose tissue weight and lipoprotein lipase activity (Wade and Heller, 1993); TAM had no effect on triiodothyronine-induced changes in food uptake (Fitts et al., 1998). The relative uterine weights in the NMU experiment were decreased, as the consequence of uterine atrophy. The parallel existence of a decrease in the body weight gain and of uterine atrophy in TAM-treated rats was also observed in other studies (Greaves et al., 1993; Kothari et al., 1997). The uterine atrophy points out to a different mechanism of TAM action in human and rat uterus. In humans, TAM could induce uterine adenocarcinomas (its precursor is endometrial hyperplasia), in rats TAM induces endometrial tumours without original endometrial hyperplasia (Carthew et al., 2000).

Many experimental studies report the cytotoxic effect of MEL in physiological concentrations in oestrogen-positive MCF-7 cell lines (Hill and Blask, 1988; Cos et al., 1996a,b); also *in vivo* studies (Kothari, 1987; Blask et al., 1991; Kothari et al., 1997) confirmed MEL as an anticarcinogenic substance. MEL administered in a single injection using the dose 500 μg per rat and day in the promotion period reduced incidence and frequency of NMU-induced mammary tumours (Blask et al., 1991). In our experiment MEL administered as water solution in the same daily entire dose as Blask et al. (1991) – about 500 μg/day (using the concentration 20 μg/ml) in late afternoon and all over the night – decreased the incidence by 30% in the NMU experiment, and reduced the tumour volume and the lengthened latency period by 12 days in the DMBA experiment. The antitumour effect of MEL was also observed in other parameters evaluated, but except for tumour volume in the DMBA experiment, the differences were not significant. In our previous experiment (Ahlersová et al., 2000), MEL was administered in even higher doses – about 2500 μg/day (concentration cca 100 μg/ml of solution) continuously all over the day. In this experiment MEL lowered only the tumour frequency, other parameters were not influenced. Kothari et al. (1997) observed a marked antitumour effect of MEL administered in the drinking water (200 μg/day) in NMU-induced mammary gland carcinogenesis in female Sprague-Dawley rats. The same author (Kothari, 1987) proved an oncostatic effect of MEL also in the dose 100 μg/day (concentration cca 4 μg/ml) when administered continuously as water solution in DMBA-induced mammary carcinogenesis in intact and pinealectomized Holtzman rats exposed to continuous light. MEL administered in the drinking water in a concentration 0.4 μg/ml in 10-month-old male Sprague-Dawley rats for 12 weeks had a prominent metabolic