doi:10.4149/neo_2009_03_252

Celecoxib and melatonin in prevention of female rat mammary carcinogenesis

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Received July 7, 2008

The present experiment aims to evaluate tumor suppressive effects of a selective inhibitor of cyclooxygenase-2 (COX-2) celecoxib (Celebrex, Pfizer) administered alone and in combination with melatonin in the prevention of N-methyl-N-nitrosourea (NMU)-induced mammary carcinogenesis in Sprague-Dawley female rats. Celecoxib was administered daily at a concentration of 1.666 g/kg diet to two groups during 20 weeks (starting a week before first NMU application). A combination of celecoxib and melatonin applied in drinking water (20 μg/ml drinking water), daily from 15:00 to 08:00 hours was administered to the second group. The anticarcinogenic effects of chemopreventive drugs were compared with control (NMU) animals. Celecoxib administration decreased mammary tumor incidence (by 24%), while combination of celecoxib and melatonin decreased tumor incidence even more significantly (-30%). Significant decrease in tumor frequency per group was recorded in both groups with chemoprevention: celecoxib alone (-54%) and combination of celecoxib and melatonin (-64%). Celecoxib significantly influenced tumor frequency per animal in the group with combination of both protective substances (-52%). Celecoxib administration resulted in prolonged latency by 3%, and by 13% in the group with combination of both protective substances. These results confirm preventive effects of celecoxib in induced rat mammary carcinogenesis. The administration of isolated MEL had only lesser effect, but in the combination with CELE revealed some potentiating influence in mammary carcinogenesis inhibition.

The present study is the first to prove efficacy of the above-mentioned celecoxib and melatonin intake. Our results point to the need for a deeper analysis of coxib efficacy in human carcinogenesis.

Key words: mammary carcinogenesis, female rats, celecoxib, melatonin

Mammary gland cancer presents a serious health issue worldwide. It is one of the most frequent malignancies in women in industrialised countries and it is the second most frequent cause of death in women with cancer. Annually, breast cancer is diagnosed in 40 000 women only in the UK [1].

Apart from maintaining a healthy lifestyle, chemoprevention is efficient to combat mammary gland cancer. Non-steroidal antiinflammatory drugs (NSAIDs) have been found to have chemopreventive effects. NSAIDs belong to the most frequently used drugs worldwide; with as many as 20 to 30 % of the population in the developed countries taking prescribed forms of these agents [2]. NSAIDs are often used in the treatment of rheumatic diseases, to alleviate pain and functional impairment.

Several retrospective clinical studies aimed at a long-term application of various NSAIDs reported significant decrease in the incidence of colon cancer [3], mammary gland cancer [4], prostate [5] and lung cancer [6]. The studies with laboratory animals proved chemopreventive effect of NSAIDs in various types of neoplasia, including mammary gland cancer. Dietary ibuprofen and celecoxib administration (one week before carcinogen application to the end of the experiment – 105 days after the application of 7,12-dimethylbenzanthracene – DMBA) in mammary tumorigenesis decreased tumor incidence, tumor frequency and tumor volume in female rats; celecoxib proved to be more efficient [7]. Nimesulide applied in a high-fat diet in rat mammary carcinogenesis induced by 2-amino-1-methyl-6-fenylimidazol (4,5-b) pyridine reduced tumor incidence, frequency and volume as opposed to the group without nimesulide [8].

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Our group proved chemopreventive effects of indomethacin administered in drinking water [9], diclofenac administered in drinking water [10], nimesulide applied subcutaneously [11] and rofecoxib applied in a laboratory diet [12] in mammary carcinogenesis in female Sprague-Dawley rats. Certain anticarcinogenic effect of etoricoxib administered in a laboratory diet [13] was recorded in female Sprague-Dawley rats.

From the pharmacodynamic point of view, COX-1 inhibitors (acetylsalicylic acid, indomethacin, ibuprofen) display serious side effects on the gastrointestinal tract associated with their long-term application. These undesirable effects are markedly reduced in administration of COX-2 inhibitors. Several recent studies pointed to an increased incidence of thromboembolic complications associated with the use of coxibs [14, 15]. Increased incidence for vascular events was reported also for long-term administration of ibuprofen and diclofenac; rofecoxib and celecoxib were associated with increased risk of myocardial infarction attributable to higher daily dose intake [15]. Discussion on possible risks of coxibs resulted in withdrawal of certain pharmaceuticals from the market in 2004. Since then the use of coxibs has gone hand in hand with criticism, fear, and skepticism. Disputes have been going on and no agreement has been reached regarding the issue of "identical" or "different" effect of NSAIDs and coxibs on cardiovascular system [16]. Targeted prospective studies with the above cited drugs may find the answers to the discussed issues.

Celecoxib belongs to the NSAIDs, which selectively inhibit COX-2. The drug (Celebrex, Pfizer) was the first specific COX-2 inhibitor used in clinical practice. It was approved by Food and Drug Administration (FDA) in the USA in 1988 for use in rheumatoid arthritis, ostheoarthritis and familial adenomatous polyposis treatment. In 2001 it was indicated to treat acute pain conditions and primary dysmenorrhoea, too.

Melatonin (5-methoxy-N-acetyltryptamine) is a pineal gland hormone, produced also in the retina and gastrointestinal tract. Antitumour effect of melatonin was observed mainly in estrogen-sensitive tumors (mammary gland and prostate tumors), but also in cervical tumors, Lewis lung carcinoma, hepatocarcinoma, melanoma and ovarian carcinoma [17, 18]. Numerous experimental studies pointed to tumor suppressive effects of melatonin via several mechanisms. Blask et al. [18] emphasised possible anti-estrogenic effect of melatonin in NMU-induced rat mammary carcinogenesis. Blask and Hill [19] points to a possible tumor suppressive effect of melatonin by inhibition of linoleic acid and its metabolite 13-hydroxyoctadecadienoic acid utilisation. Besides the antioxidative properties with free radical scavenging, MEL displays some immunomodulatory and especially antiinflammatory activities. MEL stimulates the production of IL-2, IL-6, IL-12 and depresses the effectiveness of other cytokines (interleukin-8, tumor necrosis factor-α) /review [20]/.

The present study investigates anticarcinogenic effect of celecoxib in NMU-induced mammary carcinogenesis administered alone or in combination with melatonin.

Materials and methods

Female Sprague-Dawley rats (AnLab, Prague, Czech Republic) aged 30-35 days, weighing 101-125 g were used in the experiment. The animals were adapted to standard vivarium conditions with the temperature 23±2 °C, relative humidity 60-70%, artificial regimen light : dark = 12:12 h, with lights on from 7.00 h (light intensity 150 lux per cage, light source: fluorescent lamps Tesla - 40W). During the experiment the animals drank tap water *ad libitum.* Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (NMU) (Sigma, Deisenhofen, Germany), administered intraperitoneally in two doses, each per 50mg/kg b.w., with a six-day interval between 42nd-48th postnatal days. NMU was freshly prepared by dissolving in isotonic saline solution (0.5 ml per animal). Celecoxib (Celebrex, Pfizer Pharmaceuticals Group, New York, USA) was applied in the Ssniff diet (Soest, Germany), at a concentration of 1.666 g/kg food (CELE 0,1666%). Melatonin (Sigma, Deisenhofen, Germany) was applied 3 days before carcinogen administration in drinking water at a concentration of 20 μg/ml daily from 15.00 to 8.00 (from 8.00 to 15.00 animals drank tap water). Melatonin solution was freshly prepared three times a week. Twenty mg of melatonin were diluted in 0.4 ml of 30 % ethanol and mixed up with tap water to the desired volume.

Chemoprevention with celecoxib began 7 days before carcinogen administration and lasted until the end of the experiment (20 weeks after NMU administration). The animals were divided into five groups: 1. control group, intact, without chemoprevention and carcinogen, 2. control group without chemoprevention but with carcinogen (NMU), 3. chemoprevention with celecoxib 1.666 g/kg food (CELE 0.1666%), 4. chemoprevention with melatonin (20 μg/ml of drinking water), 5. chemoprevention with celecoxib 1.666 g/kg of food (CELE 0.1666%) and melatonin (20 μg/ml of drinking water). Each group included 20 animals at the beginning of the experiment. The animals were weekly weighed and palpated in order to record the incidence, number, location and size of tumors. In the 20th week of the experiment (dated from the first NMU application), the animals were sacrificed by quick decapitation. Mammary tumors were excised, weighed, tumor size was recorded and histological typing was performed. The following basic parameters of mammary carcinogenesis were assessed in each group: 1. tumor incidence as the percentage of tumor-bearing animals in a group, 2. tumor frequency per group as the average number of tumors per group, 3. tumor frequency per animal as the average number of tumors per tumor-bearing animals, 4. average tumor volume, 5. latency period determined by the period between carcinogen administration to the appearance of the first tumor. The effect of a chemopreventive agent on the body mass gain (evaluated from the initiation until the end of the experiment), food and water intake was observed. Food and water intake during 24 h was recorded in the 5th, 12th and 19th week after carcinogen administration in 6 measurements (twice in a given week).

Tumor incidence was evaluated by Mann-Whitney U–test, other parameters by one-way analysis of variance or Kruskal-

Table 1. Chemopreventive effects of celecoxib, melatonin and their combination in NMU-induced mammary carcinogenesis in female Sprague-Dawley rats in the final 20th week of the experiment.

Group	NMU	CELE	MEL	CELE + MEL
Tumor incidence (%)	94.12	71.43 (-24%)	94.74	70.00 (-25%)
Tumor frequency per group	6.12 ±1.01	2.79 ±0.64 ♥ (-54%)	4.89 ±0.72 (-20%)	2.20 ±0.53 ♥ ♠ (-64%)
Tumor frequency per animal	6.50 ±1.00	3.90 ±0.59 ♥ (-40%)	5.17 ±0.71 (-20.5%)	3.14 ±0.34 ♥ ♠ (-52%)
Tumor volume (cm^3)	1.48 ±0.26	2.26 ±0.61 (+53%)	1.91 ±0.31 (+29%)	2.14 ±0.49 (+45%)
Tumor latency (days)	77.63 ±3.36	79.90 ±5.57 (+3%)	92.44 ±18.76 (+19%)	87.71 ±6.84 (+13%)

NMU – control group without chemoprevention, only with carcinogen (NMU),
CELE – chemoprevention with celecoxib 1.666 g/kg of diet (CELE 0.1666%),
MEL – chemoprevention with melatonin (20 μg/ml of drinking water),
CELE + MEL – chemoprevention with celecoxib 1.666 g/kg of diet (CELE 0.1666%) + melatonin (20 μg/ml of drinking water).
NMU-N-methyl-N-nitrosourea, **CELE**-celecoxib, **MEL**-melatonin
Data are expressed as means ±SEM, Significantly different: ♥ P<0,05 vs NMU; ♠ P<0,05 vs MEL; values in brackets are calculated as %-ual deviation compared to control with NMU (100%)

Table 2. Histopathological classification and number of mammary tumors.

Mammary tumors	NMU	CELE	MEL	CELE+MEL
Malignant lesions				
IC	32	12	37	13
IS-C	13	9	13	4
IS-P,C	14	4	14	-
PCA	16	4	8	4
IS-P	9	3	-	-
IC,CO	3	-	2	-
IC-SOL	-	-	1	-
I-CO	1	-	-	-
T.ACA	-	-	1	-
CS	1	-	1	-
IS-C,CO	1	-	-	-
IS-P-C-CO	1	-	-	-
IS-C,TA	-	-	1	-
IC,P	1	-	1	-
IS-CO	-	-	1	-
IC,IS	-	-	1	-
IS-P,C,FA	-	-	1	-
Benign neoplasms				
TA	3	3	4	2
FA	2	-	2	-
PAP	3	-	-	-
Total number	**100**	**35**	**88**	**23**

IC – invasive carcinoma cribriform; IS-C – ductal in situ carcinoma cribriform; IS-P,C – ductal in situ carcinoma papillary and cribriform; PCA – carcinoma papillary; IS-P – ductal in situ carcinoma papillary; IC,CO – invasive carcinoma cribriform with comedo carcinoma; IC-SOL – invasive carcinoma solid; I-CO – invasive carcinoma comedo; T.ACA – tubular adenocarcinoma; CS – carcinosarcoma; IS-C,CO – ductal in situ carcinoma cribriform and comedo; IS-P,C,CO – ductal in situ carcinoma papillary, cribriform, comedo; IS-C,TA – ductal in situ carcinoma cribriform and tubular adenoma; IC,P – invasive carcinoma cribriform and papillary; IS-CO – ductal in situ carcinoma comedo; IC,IS - invasive carcinoma cribriform and ductal in situ carcinoma; IS-P,C,FA - ductal in situ carcinoma papillary, cribriform, fibroadenoma; TA – tubular adenoma; FA – fibroadenoma; PAP – papilloma. Dominant type in mixed tumors is the first in order.

Wallis test (P<0.05 level). Tumor volume was calculated according to formula: $V\ (mm^3) = \pi \times S_1^2 \times S_2 / 12$; where ($S_1 < S_2$), S_1 is tumor width and S_2 tumor length in mm, S_1 and S_2 were measured perpendicularly. The experiment was carried out from December 2006 to May 2007.

Results

Preventive effect of celecoxib, melatonin and their combination in female Sprague-Dawley rat mammary carcinogenesis is summarised in Tab.1. A pronounced tumor suppressive effect of celecoxib applied alone was observed in tumor incidence, tumor frequency per group and animal and prolonged latency. Less marked antineoplastic effect was observed after melatonin alone and this was documented by prolonged latency and decreased tumor frequency per group and animal. More efficient tumor suppressive effect of combination of celecoxib and melatonin was found in tumor frequency per group and animal. Tumor incidence decreased non significantly by 24% in the CELE group and by 25% in the CELE+MEL group as opposed to the NMU control group. Significant decrease in tumor frequency per group by 54% and 64% was recorded in the CELE and CELE+MEL group, respectively, as opposed to the control group (NMU). Significantly decreased tumor frequency per animal by 40% was found in the CELE group and by 52% in the CELE+MEL opposed to the control. Melatonin alone non significantly decreased both parameters – tumor frequency per group and animal by 20% as opposed to the control only with NMU. Latency was prolonged non significantly by 14.8 days (+19%) in the MEL group, by 10.1 days (+13%) in the CELE+MEL group and by 2.3 days (+3%) in the CELE group when compared to the control only with NMU. Increase of average tumor volume in all the groups with chemoprevention was caused by high incidence of new tumors with small volume in the control group NMU. Invasive cribriform carcinomas and ductal in situ carcinomas prevailed in the histological typization among tumors in individual groups. The malignant/benign tumor ratio oscillated between 91% – 93% and 7% – 9%. /Tab. 2/

Daily dose of celecoxib in CELE group ranged from 149 μg to 159 μg of celecoxib/g b.w. (resp. 32.4 mg – 40.5 mg celecoxib/per animal) and 138 μg – 148 μg of celecoxib/g b.w. (resp. 32.4 mg – 35.1 mg celecoxib/per animal) in CELE+MEL group. Significant decrease in food intake was observed in the 5th week of the experiment in the group CELE (-14%) and CELE+MEL (-15%) as opposed to the control group NMU.

Significant decline in food intake was observed in the 12th week of the experiment in the group CELE+MEL when compared to the group with melatonin alone (-52%). A lower food intake was recorded only in the group with CELE+MEL (-50%) as opposed to NMU group. Changes in food intake were recorded in the 19th week of the experiment in the group CELE+MEL with a significant decrease when compared to the group with melatonin alone (-55%). Statistically significant decrease in food intake was found in CELE+MEL group (-52%) as opposed to the control only with NMU. /Tab. 4/

Daily water intake per rat in individual groups ranged from 26.48 ml to 37.34 ml in the 5th week of the experiment, from 21.25 ml to 37.92 ml in the 12th week, from 21.00 ml to 36.38 ml in the 19th week of the experiment. A decreased water intake was recorded in the group with celecoxib in the 12th week of the experiment as opposed to NMU group (-32%). Water intake in NMU was decreased by 18% when compared to intact group.

In the 19th week of the experiment, a significantly lower water intake was recorded in the group receiving celecoxib alone as opposed to NMU group (-29%). Similar effect of celecoxib on water intake was also observed in the group with combination of celecoxib and melatonin; and it was documented by significant decrease as opposed to NMU group and MEL group (-31%, -41%). /Tab. 4/

Average body weight of rats decreased in NMU group (-15%) when compared to intact group. Significant decrease in average body weight was observed in the groups CELE and CELE+MEL at the end of the experiment consistently by 7% in both groups as opposed to the control group (NMU). /Tab. 3/

Table 3. Body weight changes.

Group	Initial weight (g)	Final weight (g)	Body mass gain (g)
INT	119.75±1.31	308.75±5.59	189.00±4.28
NMU	120.75±1.68	261.77±4.85	141.02±3.17
CELE	121.05±1.41	243.43±18.98 ♥ (-7%), ♦	122.38±17.57
MEL	122.75±2.03	254.58±5.06	131.83±3.03
CELE + MEL	121.30±1.38	242.50±13.01 ♥ (-7%)	121.20±11.63

Data are expressed as means ± SEM, Significant differences : ♥ P<0,05 vs NMU; ♦ P<0,05 vs MEL; values in brackets are calculated as %-ual deviation compared to control with NMU (100%), INT – intact rats
For others: see Tab.1

Discussion

At present, medical practice prefers NSAIDs with predominant COX-1 inhibition, derivatives of arylalkanoic acids– acetic acid (e.g. diclofenac, indomethacin) and propionic acid (e.g. ibuprofen, naproxen) and oxicams (e.g. piroxicam, meloxicam); with predominant COX-2 inhibition (nimesulide), or selective COX-2 inhibition: coxibs (celecoxib, rofecoxib, valdecoxib, parecoxib, etoricoxib, lumiracoxib).

Coxibs were reported not only to have beneficial effects in locomotory apparatus diseases but also preventive effects in carcinogenesis. Clinical studies with celecoxib prevention in patients suffering from familial adenomatous polyposis reported significant decrease in colon and rectal polyps (by 28%) [21]. One of the most important retrospective studies pointed to a decreased incidence of several malignancies after 5 or more years of ibuprofen and aspirin use (100 mg and more per day), of these: 63% for colorectal cancer, 62% for gastric and 47% for ovarian cancer. Moreover, it suggests that preventive effects of NSAIDs are dose and time dependent [22]. The Harris et al. [23] study was aimed to the comparison of anticarcinogenic efficacy of selective (celecoxib, rofecoxib) COX-2 inhibitors and inhibitors with prevalent COX-1 inhibition (aspirin, ibuprofen,

Table 4. Food and water intake

	food intake (g)			water intake (ml)		
Week after NMU administration	5.	12.	19.	5.	12.	19.
INT	32.52±11.89	43.89±6.45	39.81±4.94	37.34±2.78	37.92±1.64	36.38±1.65
NMU	21.34±0.59	41.02±6.16	39.44±5.96	34.43±1.81	31.17±2.80 **a** (-18%)	30.31±2.56
CELE	18.38±0.86 **b** (-14%)	23.88±2.95	22.50±2.43	26.48±1.67	21.25±1.13 **b** (-32%)	21.50±0.80 **b** (-29%)
MEL	20.88±2.52	43.22±5.91	41.88±5.89	35.57±2.85	36.83±2.32	35.56±2.09
CELE+MEL	18.09±0.72 **b** (-15%)	20.58±3.62 **b** (-50%) **d** (-52%)	18.75±3.35 **b** (-52%) **d** (-55%)	35.00±3.06	35.83±5.39 **c** (+69%)	21.00±2.271 **b** (-31%) **d** (-41%)

Data are expressed as means ±SEM. Significant differences : [a] P<0,05 vs INT; [b] P<0,05 vs NMU; [c] P<0,05 vs CELE; [d] P<0,05 vs MEL; values in brackets are calculated as %-ual deviation compared to control with NMU (100%). INT – intact rats
For others: see Tab.1

naproxen) and found reduced risk (-70%) of mammary tumorigenesis in COX-2 inhibitor users.

Experimental studies proved chemopreventive effects of NSAIDs in various types of neoplasia, including mammary gland cancer. In McCormick et al. [24] study indomethacin administration to female Sprague-Dawley rats in a dose of 25 or 50 mg/kg diet, respectively during 207 days decreased mammary tumor incidence induced by DMBA. The experiments with indomethacin conducted by our group in DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats reported a decreased mammary tumor incidence by 63%, decreased tumor frequency per group by 88 % and prolonged latency by 59 days as opposed to control group [9]. Chemopreventive effect of diclofenac administered in drinking water to female Sprague-Dawley rats significantly decreased mammary gland tumor incidence, the frequency per group, and tumor volume [10]. Nimesulide administration with a high-fat diet in chemically induced rat mammary carcinogenesis by 2-amino-1-methyl-6-fenylimidazol[4,5-b]pyridine - PhIP reduced incidence, frequency and tumor volume as opposed to the group with high-fat diet without NIM [8]. Tumor suppressive effect of nimesulide (applied subcutaneously twice a week) was found out by our group in NMU-induced mammary carcinogenesis in female Sprague-Dawley rats [11]. No oncostatic effect of nimesulide was found in the above-mentioned experiment in DMBA-induced mammary carcinogenesis.

Our further studies were focused on preventive effects of coxibs in experimental mammary carcinogenesis. Dietary administered rofecoxib (selective COX-2 inhibitor) in NMU-induced mammary carcinogenesis in female Sprague-Dawley rats markedly decreased tumor incidence and frequency and prolonged latency period by 8 days [12]. The study focused on prevention of NMU-induced mammary carcinogenesis by etoricoxib (selective inhibitor COX-2) administered with diet to female Sprague-Dawley rats reported a tumor suppressive effect at a higher dose (0.025 mg/1 g diet) documented by a slight decrease in tumor incidence, tumor frequency per group, and prolonged latency by 7 days [13].

Anticarcinogenic effect of celecoxib documented by changes in basic parameters of carcinogenesis was observed in this study. Celecoxib administered alone and in combination with melatonin reduced tumor incidence. Pronounced decrease in tumor incidence was observed by Kubatka et. al. [12] after rofecoxib application. In our experiment, celecoxib markedly decreased tumor frequency per group and animal but increased tumor volume. Similar changes in tumor volume were found after etoricoxib application at a dose of 0.025mg/g diet [13]. As opposed to the effect of celecoxib and etoricoxib, administration of rofecoxib in the study by Kubatka et al. [12] decreased tumor volume. Celecoxib prolonged latency period as opposed to the control group only with NMU, similarly to rofecoxib [12] and etoricoxib [13]. Celecoxib reduced body weight gain per animal when compared to the control group (NMU). This may be attributed to its anorexigenic effect resulting in obvious decrease in food and water intake. These changes may be associated with a relatively high dose of celecoxib similar to that used by Harris et al. [7]. A slight decrease in body weight in the groups with rofecoxib was observed by Kubatka et al. [12] too.

Abou-Issa et al. [25] recorded inhibition of DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats by celecoxib (0.25 g/kg, 0.50 g/kg, 1.00 g/kg or 1.50 g/kg diet) administered in diet. Harris et al. [7] pointed to an obvious antitumor effect of dietary administered celecoxib (1.50 g/kg diet) in DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats.

Anticarcinogenic effect of melatonin (MEL) administered alone or in combination with another chemoprevention was investigated in our previous studies. Continuous administration of melatonin in drinking water at a concentration of 100 μg/ml during 25 weeks (melatonin was administered after DMBA application) reduced mammary tumor frequency per animal [26]. More pronounced anticarcinogenic effect was observed when MEL was administered before carcinogen application. Prolonged latency period was found out by Bojková et al. [27] in NMU-induced carcinogenesis with melatonin applied in drinking water (20 μg/ml). In the experiment carried out by Kubatka et al. [28] melatonin applied in drinking water at a concentration of 4 μg/ml markedly reduced tumor frequency (by 50%) in NMU-induced carcinogenesis. Blask et al. [29] reported decreased tumor incidence, number and size and prolonged latency period in female Sprague-Dawley rats 3 weeks after DMBA-induced carcinogenesis. The animals were administered melatonin at a concentration of 250 mg per animal during 12 weeks. Obvious anticarcinogenic effect of indomethacin and melatonin in DMBA-induced carcinogenesis was recorded in the experiment by Môciková-Kalická et al. [9]; however, the addition of melatonin did not substantially influenced the anticarcinogenic effect. No additive oncostatic effect of melatonin in combination with nimesulide on female rat mammary carcinogenesis was observed in further experiment [11]. Administration of exogenous MEL influenced the mammary carcinogenesis parameters in female SD rats induced by the chemical carcinogen (NMU) and psychoemotional stress (immobilization) [30]. Recently the inhibition of COX-2 activity by MEL was described [31]. Our data suggest rather insufficient action of isolated MEL, but the combination with a coxib (CELE) displayed an enhanced effect in mammary carcinogenesis inhibition, namely in tumor frequency reduction.

More research should be done to find the proper dose, frequency, and application method of melatonin combined with other chemopreventive substances.

This study confirmed that preventive effects of coxibs including celecoxib in experimental mammary carcinogenesis cannot be overlooked. Removal of side effects of coxibs may offer a new promise for their use in cancer prevention.

We wish to thank Pfizer Pharmaceuticals Group (New York, USA) for the generous gift of Celecoxib (Celebrex) and for financial

support. The project 1/2355/05 was supported by the Grant Science Agency – VEGA, Ministry of Education, Slovak Republic. Experiment was approved by the State Veterinary and Food Administration of Slovak Republic by accreditation No. Ro-2935/06-221.

References

[1] Mazhar D, Ang R, Waxman J et al. Cox inhibitors and breast cancer. Brit J Cancer 2006; 94: 346–350. doi:10.1038/sj.bjc.6602942

[2] Mandami M, Rochon PA, Juurlink DN et al. Observational study of upper gastrointestinal haemorrhage in elderly patients given selective cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs Brit Med J 2002; 325: 624–630. doi:10.1136/bmj.325.7365.624

[3] Thun MJ, Namboodiri MM, Heath CWJ. Aspirin use and reduced risk of fatal colon cancer, N Engl J Med 1991; 325: 1593–1596.

[4] Harris RE, Kasbari S, Farrar WB. Prospective study of non-steroidal anti-inflammatory drugs and breast cancer. Oncol Rep 1999; 6: 71–73.

[5] Nelson JE, Harris RE. Inverse association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs): results of a case-control study. Oncol Rep 2000; 7: 169–170.

[6] Harris RE, Berne-Donk J, Schuller HM. Chemoprevention of lung cancer by non-steroidal anti-inflammatory drugs among cigarette smokers. Oncol Rep 2002; 9: 693–695.

[7] Harris RE, Alshafie GA, Abou-Issa H et al. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000; 60: 2101–2103.

[8] Nakatsugi S, Ohta T, Kawamori T et al. Chemoprevention by nimesulide, a selective cyclooxygenase-2 inhibitor, of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced mammary gland carcinogenesis in rats. Jpn J Cancer Res 2000; 91: 886–892.

[9] Môciková-Kalická K, Bojková B, Adámeková E et al. Preventive effect of indomethacin and melatonin on 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in female Sprague–Dawley rats. A preliminary report, Folia Biol (Prague) 2001; 47: 75–79.

[10] Orendáš P, Kassayová M, Ahlers I et al. The chemopreventive effect of diclofenac in mammary carcinogenesis in female Sprague-Dawley rats. Proceedings of 7th Slovak Seminar in Animal Physiology, Nitra 2007, pp 243-250 / in Slovak/.

[11] Kubatka P, Kalická K, Chamilová M et al. Nimesulide and melatonin in mammary carcinogenesis prevention in female Sprague–Dawley rats. Neoplasma 2002; 49: 255–259.

[12] Kubatka P, Ahlers I, Ahlersová E et al. Chemoprevention of mammary carcinogenesis in female rats by rofecoxib. Cancer Lett 2003; 202: 131–136. doi:10.1016/j.canlet.2003.08.006

[13] Orendáš P, Ahlers I, Kubatka P et al. Etoricoxib in the prevention of rat mammary carcinogenesis. Acta Vet (Brno) 2007; 76: 613–618. doi:10.2754/avb200776040613

[14] Howard PA, Delafontaine P. Nonsteroidal anti-inflammatory drugs and cardiovascular risk, J Am Coll Cardiol 2004; 43: 519–525. doi:10.1016/j.jacc.2003.09.043

[15] Kearney PM, Baigent C, Godwin J et al. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. Brit Med J 2006; 332: 1302–1308. doi:10.1136/bmj.332.7553.1302

[16] Mitchell JA, Warner TD. Cox isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov 2006; 5: 75–86. doi:10.1038/nrd1929

[17] Sánchez-Barceló EJ, Cos S, Mediavilla MD. Influence of pineal gland function on the initiation and growth of hormone-dependent breast tumors. Possible mechanisms. In: Gupta D, Attanasio A, Reiter RJ, editors. The Pineal Gland and Cancer: Brain Research Promotion. London-Tubingen, 1988: 221–232.

[18] Blask DE, Pelletier DB, Hill SM. Pineal melatonin inhibition of tumor promotion in the N-nitroso N-methylurea model of mammary carcinogenesis: potential involvement of antiestrogenic mechanisms in vivo. J. Cancer Res. 1991; 117: 526–532. doi:10.1007/BF01613283

[19] Blask DE, Hill SM. Effects of melatonin on cancer: studies on MCF-7 human breast cancer cells in culture. J Neural Transm (Suppl) 1986; 21: 433–449.

[20] Pandi-Perumal SR, Srinivasan V, Maestroni GJM et al. Melatonin: Nature's most versatile biological signal? FEBS J 2006; 273 (13), 2813–2838. doi:10.1111/j.1742-4658.2006.05322.x

[21] Steinbach G, Lynch PM, Phillips RK et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000; 342: 1946–52. doi:10.1056/NEJM200006293422603

[22] Harris RE, Beebe-Donk J, Doss H et al. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: A critical review of non-selective COX-2 blockade. Oncol Rep 2005; 13: 559–583.

[23] Harris RE, Beebe-Donk J, Alshafie G. Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer 2006; 6: 27. doi:10.1186/1471-2407-6-27

[24] Mc Cormick DL, Madigan MJ, Moon RC. Modulation of rat mammary carcinogenesis by indomethacin. Cancer Res 1985; 45: 1803–1808.

[25] Abou-Issa HM, Alshafie GA, Seibert K et al. Dose-response effects of the COX-2 inhibitor, celecoxib, on the chemoprevention of mammary carcinogenesis. Anticancer Res 2001; 21: 3425–3432.

[26] Ahlersová E, Ahlers I, Kubatka P et al. Melatonin and retinyl acetate as chemopreventives in DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats. Folia Biol (Prague) 2000; 46: 69–72.

[27] Bojková B, Kubatka P, Môciková K et al. Effects of retinyl acetate and melatonin on N-methyl-N-nitrosourea-induced mammary carcinogenesis in rats. A Preliminary Report. Folia Biol (Prague) 2000; 46: 73–76.

[28] Kubatka P, Bojková B, Kalická K et al. Preventive effects of raloxifene and melatonin in N-methyl-N-nitrosourea-induced mammary carcinogenesis in female rats. Neoplasma 2001; 48: 313–319.

[29] Blask DE, Hill SM, Orstead KM et al. Inhibitory effects of the pineal hormone melatonin and underfeeding during the promotional phase of 7,12-dimethyl-benz(a)anthracene (DMBA)-induced mammary tumorigenesis. J Neural Transm 1986; 67: 125–138. doi:10.1007/BF01243365

[30] Kassayová M, Friedmanová L, Orendáš P et al. Effect of prolonged psychoemotional stress and melatonin on experimental mammary carcinogenesis in female rats. Neoplasma 2007; 54 (3): 251–255.

[31] Mayo JC, Sainz RM, Tan DX et al. Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. J Neuroimmunol 2005; 165: 139–149. doi:10.1016/j.jneuroim.2005.05.002