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Chemoprevention of N-Methyl-N-Nitrosourea Induced Mammary Carcinogenesis with Raloxifene and Melatonin: Metabolic Changes in Female Rats

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Abstract

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The aim of this work was to determine the selected parameters of carbohydrate and lipid metabolism in the mammary carcinogenesis induced with N-methyl-N-nitrosourea (NMU) in two doses, each by 50 mg/kg of body weight with a 7-day interval between them within the postnatal days 43 and 54 in female Sprague-Dawley rats. Chemoprevention started with the administration of melatonin (MEL, 4 μg/ml in water, from 15.00 h to 08.00 h) 12 days and raloxifene (RAL 5 mg/kg, 2 × weekly) 10 days before the application of NMU. Twenty-four weeks following the NMU administration the animals were killed, and the incidence, latency, frequency and volume of tumours were evaluated. The animals were divided into: tumour-bearing (TB) and non-tumour-bearing (NTB) with the influence of RAL, MEL and their combination. While RAL and RAL plus MEL significantly decreased the incidence and frequency of tumours, the effect of isolated MEL was substantially lower. In the serum, an increase in the concentration of serum glucose in TB and also NTB animals was observed. In the liver of both the TB and NTB animals, the content of cholesterol (CH) and triacylglycerols (TG) decreased and the contents of phospholipids (PL) increased. RAL decreased the contents of CH and PL in the liver of NTB animals and increased the concentration of TG in both groups of animals. Administration of RAL to NTB animals decreased the concentrations of malondialdehyde (MDA) in the serum and thymus, in the bone marrow also in TB animals. MEL decreased the concentration of MDA in the bone marrow of TB animals. MEL increased the concentrations of serum glucose and glycogen content in the heart muscle of NTB animals. RAL plus MEL decreased the concentration of serum TG and PL and decreased the contents of CH and PL in the liver of TB as well as NTB animals. In the thymus and liver, combination of RAL+MEL decreased the MDA content compared with the RAL alone in NTB animals.

The co-effect of two or more substances will be probably the optimal way in prevention of cancer. The co-effect of RAL and MEL shows to be a prospective way for influencing the mammary tumors.

Breast cancer, female rats, raloxifene, melatonin, chemoprevention

The hormonal therapy of the breast carcinoma is an inseparable part of the variety of therapeutic procedures. The substitution with estrogens has been considered for a long time as a dominant indication of therapy in postmenopausal women, and it has been recognized that approximately one third of women will have a benefit of this procedure. The use of estrogens protects these women against osteoporosis and decreases the cardiovascular risk, but on the other hand, increases the risk of breast and endometrium carcinoma (Col et al. 1997). With regard to the unfavourable effects of estrogens on the breast and endometrium tissue, it was necessary to develop substances which could have a favourable estrogenic effects on the bone tissue and cardiovascular system without increasing the risk of breast and endometrium carcinoma. Raloxifene (RAL) represents the second generation of the

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substances called selective modulators of estrogen receptors (SERM). RAL is a non-steroid antiestrogen with the structure of benzothiophene originally developed for the treatment and prevention of osteoporosis in postmenopausal women. Preliminary results in women treated for osteoporosis indicate that RAL reduces the risk of the incidence of breast carcinoma without an unfavourable effect on the endometrium (Blum and Cannon 1998). Both clinical and experimental studies have revealed the ability of RAL to decrease the total and LDL cholesterol in the serum. The use of RAL in hormonal therapy in women is dependent on the results of the presently running clinical studies (MORE, STAR).

The other efficient substance in the treatment of breast carcinoma is melatonin, the principal hormonal product of the pineal gland. The function of this indolamine is closely connected with the neuroendocrine, biorhythmical and immune functions (Guerrero and Reiter 1992). Numerous authors have confirmed the oncostatic effects of melatonin in the treatment of breast carcinoma (Lissoni et al. 1989) and other types of carcinoma (Blask 1993).

The aim of our work was to find the effects of raloxifene, melatonin and their combination on the selected metabolic parameters in N-methyl-N-nitrosourea induced mammary carcinoma in female Sprague-Dawley rats and to compare their effects with the metabolic effects of "classical" antiestrogen tamoxifen alone or in combination with melatonin (Chamilová et al. 2001).

Materials and Methods

Female Sprague-Dawley rats (Anlab, Prague, Czech Republic) were used in the experiment. The animals were delivered at the age of 34-38 days, and during the whole experiment they were kept under standard conditions (23 ± 2 °C, relative air humidity 60-70 %, light regimen L:D 12:12 with the beginning of the light part of the day at 07.00 h). During the experiment the animals (4-5 in a cage) were fed the PM diet (Top-Dovo, Dobrá Voda, Slovak Republic) and drank tap water *ad libitum*. Chemocarcinogen N-methyl-N-nitrosourea (NMU; Sigma, Deisenhofen, Germany) was administered to animals between the postnatal days 43 and 54 in two doses intraperitoneally, each at 50mg/kg of body weight within 7 days. Chemoprevention with raloxifene (RAL) and melatonin (MEL) started 10 days and 12 days before the administration of NMU, respectively, and lasted until the end of the experiment. RAL (LY 139481-HCl, Eli Lilly and Co., Indianapolis, USA) was administered twice a week subcutaneously in the dorsal region at a dose of 5 mg/kg of body weight of animals as 0.25 ml solution of the mixture of PEG 400: water = 1:1. The animals drank MEL (Biosynth, Staad, Switzerland) in tap water at a concentration of 4 µg/ml daily from 15.00 h to 08.00 h next day. For the preparation of 1 l solution 4 mg of MEL was dissolved in 0.3 ml of ethanol and supplemented with tap water to the volume of 1 l. During the experiment, the animals were weighed and palpated once a week, and their food and water intake was measured. At the end of the experiment in week 24, the animals were killed by a quick decapitation and subsequently the incidence and growth of mammary tumours and selected parameters of the lipid and carbohydrate metabolism were analysed. In the serum of the mixed blood, following parameters were determined: the concentration of triacylglycerols (TG), cholesterol (CH), phospholipids (PL) malondialdehyde (MDA) (as a measure of lipid peroxidation) and glucose; in the liver, the contents of TG, PL, CH, MDA and glycogen; in the bone marrow the concentrations of TG, PL, MDA; in the thymus only the content of MDA; in the heart muscle the glycogen content. The concentration of phospholipids was determined from the lipid phosphorus by Bartlett's method (1959), total cholesterol according to Zlatkis *et al.* (1953), glycogen by the method of Roe and Dailey (1966), malondialdehyde in a reaction with thiobarbituric acid (Satch 1978), triacylglycerols and glucose by the commercial sets (Lachema).

At the evaluation 9 groups were compared:
1. NMU – non-tumour-bearing animals (NTB, n = 4)
2. NMU – tumour-bearing animals (TB, n = 4)
3. NMU + RAL – non-tumour-bearing animals (NTB+RAL, n = 14)
4. NMU + RAL – tumour-bearing animals (TB+RAL, n = 3)
5. NMU + MEL – non-tumour-bearing animals (NTB+MEL, n = 6)
6. NMU + MEL – tumour-bearing animals (TB+MEL, n = 8)
7. NMU + RAL + MEL – non-tumour-bearing animals (NTB+RAL+MEL, n = 11)
8. NMU + RAL + MEL – tumour-bearing animals (TB+RAL+MEL, n = 4)
9. Intact animals (INT, n = 10)

Results were statistically evaluated by the one-way analysis of variance for $p < 0.05$.

Results

In the groups with RAL and RAL+MEL, the incidence decreased by 67 % and 65.5 %, respectively. In the groups with RAL, RAL+MEL and MEL, the frequency per group decreased by 90 %, 85 %, and by 50 %, respectively. Application of RAL lengthened the latency by 27 days in both groups. In the groups with RAL and RAL+MEL, the tumour volume was reduced, contrary to that in the group with MEL was increased. The weight gain and relative weight of the uterus decreased in RAL and RAL+MEL groups in comparison with those without RAL. In the food intake per animal and day there were no significant differences in comparison with the control group. A decrease in the food intake per animal and day was significant in the groups with RAL (RAL and RAL+MEL) in comparison with those without RAL (group with MEL and control group). A decrease in the water intake per animal and day was observed in the groups with RAL and RAL+MEL as compared with the control group (Kubatka et al. in press).

Serum

Glucose – an increase in the concentration of serum glucose was observed in both the TB and NTB animals. In NTB and TB animals, MEL increased its concentration, in NTB significantly.

Triacylglycerols – in TB animals MEL and also RAL increased the concentration of TG in the serum. Combination of RAL+MEL decreased the serum TG which were increased by RAL. In NTB animals RAL and also RAL+MEL increased the TG concentration.

Phospholipids – MEL and also RAL+MEL decreased the concentration of PL in the serum of TB animals. RAL alone and also in combination with MEL decreased the PL concentration, RAL+MEL decreased it also in comparison with MEL alone.

Cholesterol – no changes were observed.

Malondialdehyde – RAL decreased the concentration of MDA compared to its combination with MEL in TB animals. In NTB animals, combination of RAL+MEL increased the level of serum MDA that was decreased by RAL also in comparison with MEL alone.

Liver

Glycogen – combination of RAL+MEL increased the content of hepatic glycogen, also when compared to RAL and MEL alone.

Triacylglycerols – in both the TB and NTB animals there was a decrease in the content of TG. In TB animals combination of RAL+MEL decreased the content of MEL-increased TG.

Phospholipids – the PL content was increased in both the TB and NTB animals. IN TB animals RAL+MEL decreased the PL content also with regard to MEL alone. In NTB animals RAL decreased the PL content, in combination with MEL decreased it only as compared to MEL alone.

Cholesterol – in both the TB and NTB animals, the CH content was decreased. In TB animals RAL+MEL decreased the content of hepatic CH as compared to MEL. In NTB animals RAL alone and also in combination with MEL decreased the CH content.

Malondialdehyde – an increase in the MDA content was observed in both groups of animals. RAL+MEL decreased it as compared to RAL and MEL alone.

Thymus

Malondialdehyde – RAL+MEL decreased the content of MDA in NTB animals in comparison with RAL alone.

Table1
Metabolic effect of raloxifene and melatonin in the prevention of breast cancer in female Sprague-Dawley rats

	INT	TB	TB+R	TB+M	TB+R+M	NTB	NTB+R	NTB+M	NTB+R+M
Serum									
GLU [mmol/l]	4.64 ± 0.27	6.47 ± 0.32 [a]	6.14 ± 0.36	7.31 ± 0.29	7.65 ± 0.72	6.15 ± 0.55[g]	6.55 ± 0.25	7.52 ± 0.29[i]	7.07 ± 0.31
TG [mmol/l]	0.55 ± 0.09	0.42 ± 0.05	1.77 ± 0.32[b]	0.71 ± 0.11[c]	0.71 ± 0.13[de]	0,39 ± 0.12	1.23 ± 0.05[h]	0.63 ± 0.08	0.75 ± 0.06[jk]
PL [mmol/l]	2.28 ± 0.17	2.12 ± 0.15	1.78 ± 0.08	1.66 ± 0.13 [c]	1.53 ± 0.09 [d]	2.14 ± 0.030	1.57 ± 0.09 [h]	1.86 ± 0.11	1.53 ± 0.05[jl]
CH [mmol/l]	1.54 ± 0.09	1.36 ± 0.08	1.27 ± 0.09	1.25 ± 0.04	1.05 ± 0.13	1.27 ± 0.18	1.18 ± 0.05	1.21 ± 0.12	1.09 ± 0.05
MDA [mmol/l]	4.92 ± 0.23	5.31 ± 0.31	4.51 ± 0.15	5.58 ± 0.22	5.62 ± 0.26 [e]	5.63 ± 0.36	4.33 ± 0.20 [h]	4.99 ± 0.35	6.10 ± 0.24[kl]
Liver									
GLY [µmol]	39.08 ± 8.65	31.29 ± 1.81	38.862 ± 3.09	32.82 ± 2.26	34.16 ± 6.20	25.54 ± 3.06	22.85 ± 2.24	33.62 ± 3.98	48.04 ± 4.7[jkl]
TG [µmol]	338.72 ± 5.1	95.61 ± 1.2[a]	162.09 ± 6.5	180.92 ± 1.2[c]	82.91 ± 3.1[f]	110.11 ± 6.2[g]	87.2 ± 9.6	185.44 ± 6.7	104.31 ± 4.8
PL [µmol]	260.81 ± 0.9	347.71 ± 6.1 [a]	322.87 ± 6.8	324.11 ± 4.2	238.41 ± 0.6[df]	300.31 ± 0.5[g]	254.9 ± 8.1[h]	319.71 ± 9.0	257.51 ± 2.2[l]
CH [µmol]	176.5 ± 6.3	133.0 ± 4.9 [a]	110.22 ± 6.7	125.3 ± 6.5	92.6 ± 3.4[df]	118.8 ± 4.4[g]	95.3 ± 3.8[h]	127.3 ± 8.0	97.6 ± 4.5[jl]
MDA [µmol]	148.40 ± 8.61	202.41 ± 4.6[a]	264.76 ± 2.8	169.31 ± 2.1	157.22 ± 8.9	218.43 ± 8.2[g]	210.22 ± 0.4	164.0 ± 12.9	136.2 ± 4.3[jkl]
Thymus									
MDA [µmol]	3.15 ± 0.43	3.87 ± 0.45	3.93 ± 0.79	3.34 ± 0.55	2.96 ± 0.33	2.69 ± 0.24	3.57 ± 0.29	2.51 ± 0.21	2.41 ± 0.19[k]
Bone marrow									
TG [µmol/g]	75.221 ± 0.29	35.581 ± 0.5[a]	157.51 ± 1.2[b]	46.752 ± 0.32	57.521 ± 2.3 [e]	99.184 ± 3.71	63.371 ± 4.50	78.082 ± 6.13	89.601 ± 7.70
PL [µmol/g]	17.94 ± 0.63	16.52 ± 1.14	16.16 ± 1.58	22.83 ± 4.43	15.86 ± 2.86	18.32 ± 3.19	19.96 ± 1.65	25.57 ± 6.16	18.84 ± 2.56
MDA [mmol/g]	52.70 ± 5.52	77.03 ± 9.22[a]	11.33 ± 1.12[b]	45.36 ± 8.66[c]	145.91 ± 2.8[def]	85.933 ± 6.04	38.66 ± 7.15[h]	41.281 ± 3.76	196.11 ± 18.64[jkl]
Heart muscle									
GLY [µmol]	5.30 ± 0.84	3.88 ± 0.38	8.54 ± 2.42 [b]	4.63 ± 0.38	3.13 ± 0.66 [e]	3.00 ± 0.37	4.32 ± 0.31	6.13 ± 0.61[i]	4.08 ± 0.21[jl]

Data given as mean ± S.E.M., significance of differences on the level $p < 0.05$ depicted as symbol (a-l) used.

a – TB vs INT
b – TB+RAL vs TB
c – TB+MEL vs TB
d – TB+RAL+MEL vs TB
e – TB+RAL+MEL vs TB+RAL
f – TB+RAL+MEL vs TB+MEL
g – NTB vs INT
h – NTB+RAL vs NTB
i – NTB+MEL vs NTB
j – NTB+RAL+MEL vs NTB
k – NTB+RAL+MEL vs NTB+RAL
L – NTB+RAL+MEL VS NTB+MEL

INT – intact animals, TB – (NMU)-treated tumour-bearing, TB+RAL – (NMU)-treated tumour-bearing with raloxifene, TB+MEL – (NMU)-treated tumour-bearing with melatonin, TB+RAL+MEL – (NMU)-treated tumour-bearing rats with raloxifene and melatonin, NTB – (NMU)-treated non-tumour-bearing, NTB+RAL – (NMU)-treated non-tumour-bearing with raloxifene, NTB+MEL – (NMU)-treated non-tumour-bearing with melatonin, NTB+RAL+MEL – (NMU)-treated non-tumour-bearing rats with raloxifene and melatonin; GLU – glucose, GLY – glycogen, CH – cholesterol, TG – triacylglycerols, PL – phospholipids, MDA – malondialdehyde

Bone marrow

Triacylglycerols – in TB animals a decrease in TG concentration was observed in bone marrow. RAL+MEL decreased the concentration of RAL-increased TG.

Phospholipids – no changes was observed.

Malondialdehyde – in TB animals the concentration of MDA was increased. MEL and RAL alone decreased the MDA concentration, however, their combination increased it in TB animals. In NTB animals, combination of RAL+MEL also increased the concentration of MDA, which was decreased by RAL and MEL.

Heart muscle

Glycogen – in TB animals RAL+MEL decreased the content of glycogen, which was increased by RAL. In NTB animals the glycogen content was increased by MEL, in combination with RAL it was decreased. RAL+MEL increased the content of myocardial glycogen. The data are summarized in Table 1.

Discussion

The experimental carcinogenesis of mammary gland induced by chemocarcinogens DMBA or NMU is mostly dependent on the hormonal state of the host (Welsch 1985). The mammary tumours induced by NMU were confirmed to be more sensitive to estrogens and growth hormones and less sensitive to prolactin in comparison with those induced by DMBA (El-Bayoumy 1994). Hormone-dependent tumours appear to be a suitable experimental model for classification and testing the chemopreventive substances with their potential using in clinical practice. Testing the effect of individual substances with assumption of there including in human pathology, besides the effect on the tumour growth the knowledge of influencing the metabolism of the host organism is important.

The cancer disease, like the carcinogen itself, influences the metabolic patterns in the host metabolism. There are precise data analyzing the lipid metabolism in experimental and human oncology. A direct relationship between breast carcinoma and cholesterol metabolism has not been confirmed yet. However, this is not the case in the carcinoma of the colon and lung and in haematological neoplasia, where decrease in its serum concentration has been confirmed by several authors (Dessi *et al.* 1992; Rose *et al.* 1974; Spiegel *et al.* 1982). In our experiment, no changes in the serum lipids in the carcinogen (NMU)-influenced animals were observed. In the liver of the both TB and NTB animals, a decrease in CH and TG and increase in PL were observed. Within the process of tumorigenesis the content of phosphocholine fraction of PL: phosphatidylcholine and phosphatidylethanolamine increases in the epithelial cells of the mammary gland (Aboagye and Bhujwalla 1999). In this connection, it is important to accent the finding of increased content of PL in the liver of TB and NTB animals in our experiment. In the liver of TB and NTB animals, an increased content of MDA was observed, which confirms the increase in peroxidation of lipids by the influence of the carcinogen as well as the cancer itself. The carbohydrate metabolism of the organism with tumour is characterized, above all, by the increased glucogenesis. In TB and NTB animals an increased concentration of serum glucose was observed.

Raloxifene belongs to the selective modulators of estrogen receptors and displays estrogen-agonistic effect to bones and lipid metabolism and estrogen-antagonistic effect on the uterine mucosa and breast tissue (Scott *et al.* 1999). Previous studies showed that RAL reduces the risk of breast carcinoma in women treated for osteoporosis without an unfavourable effect on the endometrium (Blum and Cannon 1998). RAL decreased LDL cholesterol, but did not influence the concentration of HDL cholesterol and triacylglycerols

in the serum of patients with breast carcinoma (Delmas et al. 1997). Decrease in insulin growth factor-1 and in serum insulin/glucose ratio was noted in nondiabetic postmenopausal women, treated for osteoporosis by raloxifene (Oleksik et al. 2001). Serum non-HDL cholesterol and the index apolipoprotein-B/apolipoprotein-A1 were lowered in healthy postmenopausal women, receiving daily 60mg of raloxifene; the same effect was gained by hormone replacement therapy (Anderson et al. 2001). We confirm that RAL reduced the content of CH and PL in the liver in NTB animals; in the serum its effect on CH was not observed. In contrary, RAL increased the TG concentrations in both groups. Due to the effect of RAL, there was a decrease in the concentration of MDA in the serum and thymus (in combination of RAL+MEL) in NTB animals and in the bone marrow in both groups of animals.

In many experiments MEL showed the ability to inhibit the breast tissue of spontaneous but mainly in chemically induced tumours. A full oncostatic effect has been proved under the *in vitro* conditions in the cells of mammary and prostatic carcinomas, whose growth is hormone-dependent (review: Cos and Sánchez-Barceló 2000). Cos et al. (1996) have confirmed a direct inhibitory effect of MEL on the proliferation of human MCF-7 breast carcinoma cells, RL95-2 of endometrium carcinoma and SK-N-SH of neuroblastoma cell lines. MEL significantly lengthens duration of the cell cycle of human breast carcinoma cells. MEL added into the cultivation medium together with [^{3}H] thymidine shifts the period of the MCF-7 cell cycle from 20.36 to 23.48 h. There are few data about the effect of MEL on the host metabolism. A decrease in the serum CH and inhibition of lipogenesis in the adipocytes of healthy rats were described by Vaughan and Vaughan (1993). In TB animals, the application of MEL increased the concentration of TG and decreased the concentration of PL in the serum and decreased the concentration of MDA in the bone marrow. MEL significantly influences also the carbohydrate metabolism. Several authors have demonstrated that MEL can suppress the release of insulin, while pinealectomy decreases the concentration of serum glucose (Quay and Gorray 1980). Bailey et al. (1974) confirmed the inhibition of glucose-induced secretion of insulin by MEL. Depressive effect of intracerebrally administered MEL on hyperglycaemia induced by 2-deoxyglucose has been proved (Shima et al. 1997). MEL increased the concentration of serum glucose and content of glycogen in the myocardium of NTB animals in our experiment; this should be connected with a reduced effect of insulin on the periphery.

With regard to the efficacy of two chemoprotective substances used, the combination was more expressive than their individual application. Their combination decreased the RAL-increased concentration of serum TG and decreased the concentration of PL in the serum of both groups of animals. A decrease in the content of CH and PL in the liver was observed in TB as well in NTB animals; in the liver of TB animals due to combination a decrease in MEL-increased content of TG occurred. Different results were observed in the concentration /content of MDA. In the serum, RAL+MEL increased the concentration of MDA as compared to RAL alone; in the bone marrow increased it in both groups of animals. In the thymus and liver, the combination decreased the MDA content as compared to RAL alone in NTB animals.

More studies have confirmed that tamoxifen, unlike raloxifene, increases the concentration of serum TG in breast carcinomas (Kanel et al. 1997). In our experiment, the rise of serum TG under the influence of RAL was noted in TB and NTB rats, but a decrease occurred after combined RAL+MEL treatment. Hozumi et al. (2000) compared the effect of RAL and TAM in the lipid metabolism under the *in vitro* conditions. They concluded that RAL and TAM significantly reduced the intracellular concentration of CH in HepG2 cells and TAM can increase the serum TG and so can participate in fatty liver induction in sensitive patients. These authors consider RAL a safer component in the therapy of the patients with unstable level of TG or with predisposition to hypertriacylglycerolemia.

In DMBA-induced mammary carcinogenesis, an increase in the concentration of TG in the serum was observed after application of tamoxifen; contrariwise after induction with NMU a decrease of TG was recorded (Chamilová et al. 2001). In this experiment RAL, at variance with described results, increased the concentration of TG in the serum. In both experiments with TAM, its effect manifested in a decrease in the concentration of serum CH, in NMU also in a reduction of CH content in the liver. RAL did not have an effect on the serum CH, however, reduced its content in the liver.

The effort to find the most suitable drug for cancer prevention has led us to select and combine substances that are more efficient so that the best results would be achieved. Co-effect of two or more substances will be probably the optimal way in the therapy but also in prevention of cancer. The co-effect of RAL and MEL show to be a prospective method in experimental breast cancer prevention.

Chemoprevencia raloxifénom a melatonínom u N-metyl-N-nitrózoureou indukovanej mamárnej karcinogenézy: metabolické zmeny u samíc potkanov

Cieľom práce bolo stanoviť vybrané ukazovatele sacharidového a lipidového metabolizmu u mamárnej karcinogenézy indukovanej N-metyl-N-nitrózoureou (NMU) v dvoch dávkach, každá po 50mg/kg hmotnosti medzi 43.-54. postnatálnym dňom u samíc potkanov Sprague-Dawley. Chemoprevencia začala aplikáciou melatonínu (MEL, 4 µg/ml vo vode, cirkadiánne) 12 dní a raloxifénu (RAL, 5 mg/kg 2x týždenne) 10 dní pred aplikáciou NMU. Po 24 týždňoch od podania NMU boli zvieratá usmrtené, bola hodnotená incidencia, latencia, frekvencia a objem nádorov. Zvieratá sme rozdelili do dvoch veľkých častí: nádorové (TB) a nenádorové (NTB), s ovplyvnením RAL, MEL a ich kombináciou. Zatiaľ čo RAL a RAL plus MEL významne znížili incidenciu a frekvenciu nádorov, účinok izolovaného MEL bol podstatne menší. V sére sme pozorovali zvýšenie koncentrácie sérovej glukózy u TB aj NTB zvierat. V pečeni TB aj NTB zvierat sa znížil obsah cholesterolu (CH) a triacylglycerolov (TG) a zvýšil obsah fosfolipidov (PL). RAL znížil obsah CH a PL v pečeni NTB zvierat, zvýšil koncentráciu TG u TB aj NTB zvierat. Podanie RAL u NTB zvierat znížilo koncentráciu malonyldialdehydu (MDA) v sére a týmuse, v kostnej dreni aj u TB zvierat. MEL znížil koncentráciu MDA v kostnej dreni TB zvierat. MEL zvýšil koncentráciu sérovej glukózy a obsah glykogénu v myokarde NTB zvierat. RAL+MEL znížili koncentráciu sérových TG a PL a znížili obsah CH a PL v pečeni TB aj NTB zvierat. V týmuse a pečeni kombinácia látok znížila obsah MDA oproti samotnému RAL u NTB zvierat.

Spolupôsobenie dvoch, resp. viacerých substancií bude pravdepodobne optimálnou cestou v prevencii nádorových ochorení. Spoločné pôsobenie RAL a MEL sa ukazuje ako perspektívny spôsob ovplyvnenia nádorového ochorenia mliečnej žľazy.

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