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Cancer Letters 163 (2001) 51–57

CANCER Letters

www.elsevier.com/locate/canlet

Effect of light/dark regimen on *N*-nitrosoethylurea-induced transplacental carcinogenesis in rats

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Received 20 August 2000; received in revised form 27 October 2000; accepted 28 October 2000

Abstract

Pregnant females were randomly subdivided into three groups (24 rates per group) and kept at the 12:12 h light/dark regimen (group 1), at the constant light illumination (24 h a day, group 2) or at the continuous darkness (group 3). *N*-nitrosoethylurea (NEU) has been injected into the tail vein of all rats (80 mg/kg) on the 18–19th day of the pregnancy. After the delivery the lacting dams and their progeny during the lactation period (1 month after delivery) were kept also at the three different light/dark regimens. Then all offspring from each group was kept at the 12:12 h light/dark regimen, males and females separately, and were observed until natural death.. The exposure to constant light significantly promoted the transplacental carcinogenesis whereas the exposure to constant darkness inhibited it. The incidence of total tumors, tumors of both a peripheral nervous system and kidney was 2.6; 2.5 and 8.5 times higher, and survival significantly shorter, correspondingly, in rats from the group 2 exposed to the constant light regimen as compared to the group 1 (12:12 h light/dark regimen) ($P < 0.05$). On the other hand, the exposure to the continuous darkness during the pregnancy and the lactation period significantly inhibited the transplacental carcinogenesis in the offspring of rats treated with NEU. The incidence of total tumors, tumors of a peripheral nervous system was by 2.4 and 2.7 times less, and survival longer, respectively, in exposed to the darkness rats from the group 3 as compared to the group 1 (12:12 h light/dark regimen) ($P < 0.05$). Thus, our data firstly have shown the modifying effect of light-dark regimen on the realization of the transplacental carcinogenesis induced by NEU in rats.

Keywords: Light/dark regimen; Transplacental carcinogenesis; *N*-nitrosoethylurea, Rat

1. Introduction

It is well known that many mammalian biological functions are modulated by circadian rhythms which are endogenous and genetically based [1–3]. They are driven by the hypothalamic suprachiasmatic nucleus which through multisynaptic pathway regulates circadian rhythm of synthesis and secretion of melatonin, a hormone produced by the pineal gland [1]. Melatonin is produced during night time and influences the circadian and seasonal timing of a variety of physiological and behavioral processes. It was shown that melatonin inhibits carcinogenesis in the mammary gland, colon, endometrium and uterine cervix of laboratory rodents [4–9]. Light entrains melatonin rhythm by supressing its synthesis during the day [1,10]. An exposure to the

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0304-3835/01/$ - see front matter
PII: S0304-3835(00)00673-X

light-at-night inhibits the synthesis and secretion of melatonin [11,12]. Long-term exposure to the constant light (24 h/day) enhances the development of transplantable, spontaneous and induced by 7,12-dimethyl-benz(*a*)anthracene (DMBA) or *N*-nitrosomethylurea-induced mammary tumors as well as *N*-nitrosodiethylamine-induced hepatocarcinogenesis [11–22] whereas the light deprivation by the exposure to the continuous darkness or blinding inhibited their growth and development [4,6,13,23–25]. The light deprivation has potentiated the efficacy of hormone- and chemotherapy of breast carcinoma both in rodents and women [26–28].

Experimental studies have shown that various *N*-nitroso compounds, polycyclic aromatic hydrocarbons and aromatic amines are potent transplacental carcinogens [29]. There are no available data on the effect of light/dark regimen during a pregnancy and a lactation on the development of tumors in animals exposed to a carcinogen in utero.The present study investigated the effect of various light/dark regimes on the realisation of the transplacental carcinogenesis induced by a potent carcinogen-*N*-nitrosoethylurea (NEU). We hypothesized that the constant light exposure would exert a promoting effect on NEU-induced carcinogenesis whereas the continuous darkness would be an inhibitory effect.

2. Materials and methods

2.1. Animals

Female and male Wistar-derived outbred rats were from our breeding department. Before mating they kept separately under the 12 h light:12 h dark regimen, temperature 22 ± 2°C and received standard lab chow and tap water ad libitum. For a mating two females were put together with one male. Pregnancy was registered when vaginal plug has been observed. Then pregnant females were randomly subdivided into three groups (24 rats per group) and kept in three different light-dark regimens. Group 1 was kept under the 12:12 h light/dark regimen and served as the control group. Rats from group 2 were kept in the room with the constant light regimen. Rats from group 3 were maintained in the full darkness. Dim red light was switched on in the room only during nutrition, care and service manipulations.

2.2. Carcinogen

N-nitrosoethylurea (NEU) was synthesized in the Laboratory of Organic Synthesis, N.N. Petrov Research Institute of Oncology, St. Petersburg, Russia, was 99% pure and kept at the temperature −20°C.

2.3. Experiment

Before treatment the carcinogen was dissolved in sterile normal saline and during 1 h under a light ether anaesthesia had been injected into the tail vein of all rats (80 mg/kg) on the 18–19th day of the pregnancy. Just for the injection of the carcinogen the pregnant rats from the group 3 were taken on the period not longer than 10 min in illuminated room. All pups were kept with their dams during the lactation period. Thus, pregnant and lacting dams and their progeny during the lactation period (1 month after delivery) were kept in the three different light/dark regimens. Then all offsprings from each group were taken into different cages (with five to seven animals in each) in the 12:12 h light/dark regimen, males and females separately. All animals were under observation until natural death or were sacrificed by ether vapour when moribund.

2.4. Pathology examination

Full autopsy has been performed on each animal. All main peripheral nerves and plexuses were carefully prepared and examined at autopsy. Tumors discovered at autopsy as well as some tissues suspected for tumor growth were fixed in a neutral formaline. Histological sections 5–7 μm thick were stained with hematoxilin-eosin and pycrofuxine according to Van-Gison. All tumors were classified according to the classification of the International Agency for Research on Cancer [30].

2.5. Statistics

The statistical treatment of the results was performed using Fischer exact, chi-square and Student *t*-tests [31].

3. Results

The data on the frequency and localization of transplacental tumors developed in rats exposed to NEU in utero as well as on their survival are shown in Table 1 and Fig. 1. The light/dark regimen significantly modified the transplacental carcinogenesis induced by NEU in rats. The exposure to the constant light illumination promoted it whereas the exposure to the constant darkness inhibited the carcinogenesis. The incidence of total tumors, tumors of both a peripheral nervous system and kidney was 2.6; 2.5 and 8.5 times higher, and survival significantly shorter, correspondingly, in rats from the group 2 exposed to the constant light as compared to the group 1 (12:12 h light/dark regimen) ($P < 0.05$). On the other hand, the exposure to the continuous darkness during the pregnancy and the lactation period significantly inhibited the transplacental carcinogenisis in the offspring of rat treated with NEU (Table 1). The incidence of total tumors, tumors of a peripheral nervous system was 2.4 and 2.7 times less, and survival longer, correspondingly, in rats from group 3 exposed to the constant darkness as compared to group 1 (12:12 h light/dark regimen) ($P < 0.05$). There was no difference in the incidence of kidney tumors between groups 1 and 3.

The histopathological examination has shown that the major localization of tumors were perypheral nerves and kidneys. All kidney tumors were classified as mesenchymal tumors [30]. Among 17 kidney tumors, ten were localized in the right and seven in the left kidney. Twenty-eight tumors of other localization were observed. There were seven brain tumors (six in group 2 and one in group 1), 15 mammary tumors (ten fibroadenomas and two adenocarcinomas in group 2 and three fibroadenomas in group 1) and six skin papillomas in group 1.

The distribution of the nervous system tumors by localization and histological type is presented in Table 2. Neurogenic tumors were localized in the nervus ischiadicus (37 cases), in the sacrolumbar plexus (22 cases), in the cervical plexus (21 cases) and in the nervus trigeminus (six cases). Macroscopically, tumors, as a rule, were connected with nerve trunks, were oval or oblong, glittering, greyish-white at the section. Their sizes usually fluctuated from 0.5 to 5 cm in diameter. The lighting conditions influenced significantly the morphological spectrum of induced tumors. Malignant tumors of perypheral nervous system were found in animals maintained at the constant light regimen four times as often as compared with control group (25 cases in group 2 and six cases in group 1). On the other hand, only benign neurinomas were observed (seven cases) when animals were maintained in the darkness (group 3).

At the microscopic examination of 86 neurogenic tumors, 20 were classified as neurogenic sarcomas,

Table 1
Tumor incidence and localization in rats kept under different light/dark regimen[a]

Group	Light/dark regimen	Sex	Number of rats at the end of lactation	Number of tumor-bearing rats			Mean survival of tumor-bearing rats (days)	
				Total (%)	Peripheral nervous system (%)	Kidney (%)	Peripheral nervous system	Kidney
1	12:12	Male	61	16 (26.2)	12 (19.7)	1 (1.6)	366 ± 39.7	396 ± 33.7
		Female	66	21 (31.8)	17 (27.9)	1 (1.5)	366 ± 39.7	396 ± 33.7
		Total	127	37 (29.1)	29 (22.8)	2 (1.6)	366 ± 39.7	396 ± 33.7
2	24 h light	Male	34	29 (85.3)**	21 (61.8)**	7 (20.6)**	176 ± 27.5**	275 ± 31.6*
		Female	54	38 (70.4)**	29 (53.7)**	5 (9.3)	176 ± 27.5**	275 ± 31.6*
		Total	88	67 (76.1)**	50 (56.8)**	12 (13.6)**	176 ± 27.5**	275 ± 31.6*
3	24 h darkness	Male	40	5 (12.5)**	4 (10.0)	1 (2.5)	505 ± 49.2*	527 ± 46.2*
		Female	44	5 (11.4)**	3 (6.8)*	2 (4.5)	505 ± 49.2*	527 ± 46.2*
		Total	84	10 (11.9)**	7 (8.3)**	3 (3.6)	505 ± 49.2*	527 ± 46.2*

[a] The difference with the parameter in the group 1 is significant: *, $P < 0.05$; **, $P < 0.01$.

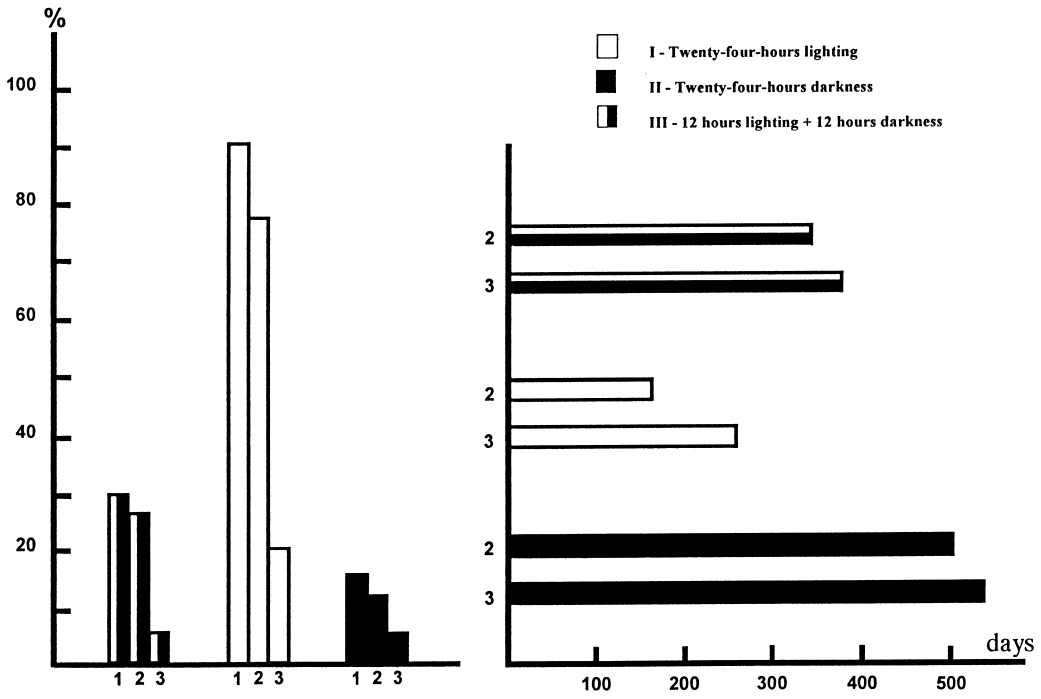

Fig. 1. The influence of light regimen on frequency and mean latency period of transplacental tumors development. (1) Neoplasms of all localizations; (2) peripheral nervous system tumors; (3) kidney tumors.

and 11 as neuroblastomas. Among benign tumors reticular neurinomas (31 cases) were observed more often than fascicular neurinomas (24 cases). Characteristic structures, so called Verokay bodies, were revealed in fasciular neurinomas. In six cases the residual ganglion cells have been observed in fascicular neurinomas.

4. Discussion

The results of our study give an evidence that the exposure to the constant light during the pregnancy and the lactation potentiates the realization of the transplacental carcinogenesis induced by NEU in rats whereas the exposure to the continuous darkness inhibits it. Our data are in agreement with other observations on the stimultating effect of the constant light regimen on mammary carcinogenesis induced by DMBA, *N*-nitrosomethylurea or *N*-nitrosodiethylamine [7,11,14,16–20]. It was shown that the exposure to constant light was followed by the inhibition of the night secretion of melatonin in humans, rats and Syrian hamsters [1,10,12,13] whereas the serum level of prolactin was increased [4,12]. It was shown that the exposure to the continuous darkness decreased the serum level of follicle-stimulating hormone, estradiol-17β and 11-oxicorticosteroids in female rats [26] as well as decreased the mitotic activity and the growth rate of transplantable mammary carcinoma RMK-1 in rats [25].

The available data have shown that the inhibition of the pineal gland function induced by pinealectomy, gangliectomy of upper cervical ganglion or by the exposure to a constant light regimen was followed by stimulation of carcinogenesis induced by chemical carcinogens whereas the administration of melatonin or the pineal peptide preparation epithalamin inhibits it [4,7,12,13,32–34]. The treatment with epithalamin started at the age of 2 months of rats exposed in utero to NEU was followed by inhibition of the development of nervous system and kidney tumors [35]. It is worthy of note that administration of epithalamin induced the increase of the night synthesis of melato-

Table 2
Localization and type of neurogenic tumors in rats transplacentally exposed to different light–dark regimen

Localization	Sex	Histological type of tumors												
		Benign tumors						Malignant tumors						Total tumors
		Fascicular neurinoma			Reticular neurinoma			Tumor of peripheral nervous system			Neuroblastoma			
		Groups[a]												
		1	2	3	1	2	3	1	2	3	1	2	3	
N. ischiadicus	Male	1	1	1	3	4	1	1	3	–	–	–	–	15
	Female	3	2	–	5	4	2	2	4	–	–	–	–	22
	Total	4	3	1	8	8	3	3	7	–	–	–	–	37
Cervical plexus	Male	1	2	1	1	1	–	–	2	–	–	2	–	10
	Female	1	3	–	2	1	–	1	1	–	–	2	–	11
	Total	2	5	1	3	2	–	1	3	–	–	4	–	21
Sacrolumbar plexus	Male	–	1	–	1	–	–	2	1	–	–	3	–	8
	Female	2	3	–	–	1	1	–	3	–	–	4	–	14
	Total	2	4	–	1	1	1	2	4	–	–	7	–	22
Gasser node of *N. trigeminus*	Male	1	–	–	–	1	–	–	–	–	–	–	–	2
	Female	–	1	–	2	–	1	–	–	–	–	–	–	4
	Total	1	1	–	2	1	1	–	–	–	–	–	–	6
Total per group	Male	3	4	2	5	6	1	3	6	–	–	5	–	35
	Female	6	9	–	9	6	4	3	8	–	–	6	–	51
	Total	9	13	2	14	12	5	6	14	–	–	11	–	86
Total	Male	9	9	9	9	12	12	12	9	9	9	5	5	35
	Female	15	15	15	15	19	19	19	11	11	11	6	6	51
	Total	24	24	24	24	31	31	31	20	20	20	11	11	86

[a] 1, 12:12 h light/dark regimen; 2, 24 h light; 3, 24 h darkness.

nin in the pineal gland and secretion of the hormone into the blood in rats [32]. It was shown, that melatonin is a potent antimutagen [9,36–38] and antioxidant [39,40]. Recently it was observed that pinealectomy before pregnancy was followed by an increase in lipid peroxidation in tissues of non-pregnant and pregnant rats after delivery of their youth whereas melatonin inhibited it [41]. The authors suggested that during pregnancy high levels of oxidative stress induce an increase in oxidative damage to lipids, which is inhibited by the antioxidative action of melatonin. Our data suggest that the exposure to the constant light regimen inhibits the level of melatonin that followed by decrease in its antioxidant and antimutagenic effect in pregnant and lactating rats, whereas the exposure to the continuous darkness leading to increase in the level of melatonin in the organism thus inhibits mutagenic and carcinogenic effect of NEU. We have found that melatonin inhibited mutagenic effect of *N*-nitroso compounds in vivo and in vitro [9,36,37].

Thus, our data firstly have shown the modifying effect of light-dark regimen on the realization of the transplacental carcinogenesis induced by NEU in rats. It is possible to suggest that the decrease of the level of melatonin induced by the exposure to the constant light plays a significant role in the stimulating effect of the constant light regimen on the transplacental carcinogenesis.

References

[1] J. Arendt, Melatonin and the Mammalian Pineal Gland, Chapman & Hall, London, UK, 1995.

[2] M. Hastings, E.S. Maywood, Circadian clocks in the mammalian brain, Bioessays 22 (2000) 23–31.

[3] K. Wager-Smith, S.A. Kay, Circadian rhythm genetics: from flies to mice to humans, Nat. Genet. 26 (2000) 23–27.

[4] V.N. Anisimov, I.G. Popovich, M.A. Zabezhinski, Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal tumors induced by 1,2- dimethylhydrazine in rats, Carcinogenesis 18 (1997) 1549–1553.

[5] V.N. Anisimov, M.A. Zabezhinski, I.G. Popovich, E.A. Zaripova, S.A. Musatov, V. Andre, C. Vigreux, T. Godard, F. Sichel, Inhibitory effect of melatonin on 7,12-dimethylbenz[a]anthracene- induced carcinogenesis of the uterine cervix and vagina in mice and mutagenesis in vitro, Cancer Lett. 156 (2000) 199–205.

[6] D.E. Blask, in: H.S. Yu, R.J. Reiter (Eds.), Melatonin in oncology, in Melatonin. Biosynthesis, Physiological Effects and Clinical Applications, CRC Press, Boca Raton, FL, 1993, pp. 447–475.

[7] S. Cos, E.J. Sanchez-Barcelo, Melatonin and mammary pathological growth, Front. Neuroendocrin. 21 (2000) 133–170.

[8] F. Deerberg, C. Bartsch, G. Pohlmeyer, H. Bartsch, Effect of melatonin and physiological epiphysectomy on development of spontaneous endometrial carcinoma in BDII/HAN rats, Cancer Biother. Radiopharmacol. 12 (1997) 420.

[9] S.A. Musatov, V.N. Anisimov, V. André, C. Vigreux, T. Godard, F. Sichel, Effects of melatonin on *N*-nitroso-*N*-methylurea-induced carcinogenesis in rats and mutagenesis in vitro (Ames test and COMET assay), Cancer Lett. 138 (1999) 37–44.

[10] G.C. Brainard, A.J. Lewy, M. Menaker, Dose response relationship between light irradiance and suppression of plasma melatonin in human volunteers, Brain Res. 454 (1988) 212–218.

[11] V.N. Anisimov, O.V. Zhukova, D.Sh. Beniashvili, V.G. Bilanishvili, M.Z. Menabde, Light deprivation, electromagnetic fields and mammary carcinogenesis, Adv. Pineal Res. 7 (1994) 229–234.

[12] R.G. Stevens, B.W. Wilson, L.A. Anderson (Eds.), The Melatonin Hypothesis. Breast Cancer and Use of Electric Power Batelle Press, Columbus, OH, 1997.

[13] V.N. Anisimov, R.J. Reiter, Pineal gland function in cancer and aging, Vopr. Onkol. 36 (1990) 259–268.

[14] J.A. Bell, A. Sneddon, T. Hamilton, Influence of light and 9,10-dimethylbenz(a)anthracene on rat ovarian steroidogenesis: neutral steroids, Biochem. J. 110 (1968) 29–30.

[15] G.C. Brainard, R. Kavet, L.I. Kheifets, The relationship between electromagnetic field and light exposures to melatonin and breast cancer risk: a review of the relevant literature, J. Pineal Res. 26 (1999) 65–100.

[16] T. Hamilton, A. Sneddon, Environmental light and DMBA-induced rat mammary tumours, Br. J. Surg. 55 (1968) 76.

[17] S. van den Heiligenberg, P. Depres-Brummer, H. Barbason, B. Claustrat, M. Reynes, F. Levi, The tumor promoting effect of constant light exposure on diethylnitrosamine-induced hepatocarcinogenesis in rats, Life Sci. 64 (1999) 2523–2534.

[18] J.W. Jull, The effect of infection, hormonal environment, and genetic contstitution on mammary tumor induction in rats by 7,12-dimethylbenz(a)anthracene, Cancer Res. 26 (1966) 2368–2373.

[19] I.K. Khaetsky, Effect of hypothalamo-pituitary disturbances induced by constant light on the level of induced mammary tumors in rats, Vopr. Exp. Onkol. (Kiev) 1 (1965) 87–93.

[20] L. Kothari, P.N. Shah, M.C. Mhatre, Effect of continuous light on the incidence of 9,10-dimethyl-1,2-benzanthracene induced mammary cancer in female Holtzman rats, Cancer Lett. 16 (1982) 313–317.

[21] N.I. Lazarev, E.A. Ird, I.O. Smirnova, Experimental Models of Endocrine Gynaecologic Diseases, Meditsina, Moscow, 1976.

[22] B.J. Smyth, A. Sneddon, T. Hamilton, Influence of light and 9,10-dimethylbenz(a)anthracene on rat ovarian steroidogenesis: phenolic steroids, Biochem. J. 110 (1968) 28–29.

[23] M.P. Coleman, R.J. Reiter, Breast cancer, blindness and melatonin, Eur. J. Cancer 28 (1992) 501–503.

[24] R.A. Hahn, Profund bilateral blidness and the incidence of breast cancer, Epidemiology 2 (1991) 208–210.
[25] A.K. Kuralasov, Effect of dark adaptation on carcinogenesis and efficacy of treatment of mammary tumor induced by DMBA, Izvestia of the Kazakh SSR Acad. Sci., Ser. Biol. 4 (1979) 79–82.
[26] A.K. Kuralasov, Modifying effect of darkness on growth of mammary carcinoma and efficacy of hormonotherapy in experiment, Bull. Exp. Biol. Med. 7 (1975) 82–84.
[27] A.K. Kuralasov, V.V. Fomina, Influence of light-dark regimen on growth and efficacy of chemotherapy of mammary carcinoma, Izvestia of the Kazakh SSR Acad. Sci., Ser. Biol. 5 (1983) 54–56.
[28] A.K. Kuralasov, V.V. Fomina, On new approaches to increasing of effectiveness of tamoxifen in treatment of breast cancer, Izvestia of the Kazakh SSR Acad. Sci., Ser. Biol. 4 (1986) 67–70.
[29] N.P. Napalkov, J.M. Rice, L. Tomatis, H. Yamasaki (Eds.), Perinatal and Multigeneration Carcinogenesis, IARC Sci. Publ. No. 96, IARC, Lyon, 1989.
[30] V.S. Turusov, U. Mohr U (Eds.), Pathology of Tumours in Laboratory Animals. Volume I-Tumours of the Rat, 2nd ed., IARC Sci. Publ., No. 99, IARC, Lyon, 1990.
[31] J.J. Gart, D. Krewski, P.N. Lee, J. Wahrendorf (Eds.), Statistical Methods in Cancer Research, Vol. 3- The Design and Analysis of Long-term Animal Experiments IARC Sci Publ No 79IARC, Lyon, 1986.
[32] V.N. Anisimov, V.Kh. Khavinson, V.G. Morozov, Twenty years of study on effect of pineal peptide preparation: epithalamin in experimental gerontology and oncology, Ann. N. Y. Acad. Sci. 719 (1994) 483–493.
[33] C. Aubert, P. Janiaud, J. Lecalvez, Effect of pinealectomy and melatonin on mammary tumor growth in Sprague–Dawley rats under different conditions of lighting, J. Neural Transm. 47 (1980) 121–130.
[34] C. Bartsch, H. Bartsch, T.H. Lippert, The pineal gland and cancer: facts, hypotheses and persrectives, Cancer J. 3 (1992) 194–199.
[35] V.G. Bespalov, V.G. Alexandrov, Effect of anti-carcinogenic drugs on an transplacental carcinogenic effect of NEU, Bull. Exp. Biol. Med. 7 (1985) 73–76.
[36] S.A. Musatov, V.N. Anisimov, V. André, C. Vigreux, T. Godard, F. Sichel, Modulatory effects of melatonin on genotoxic response of reference mutagens in the Ames test and the COMET assay, Mutat. Res. 417 (1998) 75–84.
[37] S.A. Musatov, S.V. Rosenfeld, E.F. Togo, V.S. Mikheev, V.N. Anisimov, The influence of melatonin on mutagenicity and antitumor action of cytostatic drugs in mice, Vopr. Onkol. 43 (1997) 623–627.
[38] Vijalaxmi, M.L. Meltz, R.J. Reiter, T.S. Herman, Melatonin and protection from genetic damage in blood and bone marrow: whole-body irradiation studies in mice, J. Pineal Res. 27 (1999) 221–225.
[39] R.J. Reiter, Antioxidant actions of melatonin, Adv. Pharmacol. 38 (1997) 103–117.
[40] R.J. Reiter, D.X. Tan, J. Cabrera, D. D'Arpa, R.M. Sainz, J.C. Mayo, S. Ramos, The oxidant/antioxidant network: role of melatonin, Biol. Signals Recept. 8 (1999) 56–63.
[41] R.M. Sainz, R.J. Reiter, J.C. Mayo, J. Cabrera, D.X. Tan, W. Qi, J.J. Garcia, Changes in lipid peroxidation during pregnancy and after delivery in rats: effect of pinealectomy, J. Reprod. Fertil. 119 (2000) 143–149.