HORMONE SECRETED BY THE PINEAL GLAND — MELATONIN FEEDSIDEWARD INVOLVEMENT IN CANCER GROWTH

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Here the presence of a chronomodulating actions of melatonin via *feedsideawards* mechanism *in vitro* as well as, *in vivo*, in two experimental models was presented. Intriguing process of how this takes place may due to an interacting pineal pituitary-adrenal networks (1). *In vivo* studies confirmed the role of melatonin in the study on Meth-A-sarcoma in mice and in LOU tumor growth. Melatonin disrupted circadian time structure of *in vivo* tumor growth on a *feedsideawards* manner in the case of the immunocytoma growth in female inoculated rats. Low doses disrupted the circadian DNA synthesis of mF-cells. Drug delivery systems must be taken in consideration the role of melatonin *feedsideawards* involvement during chronomodulated therapy of cancer patients.

Key words: melatonin, *feedsideward*, chronomodulating actions, pineal pituitary-adrenal networks, LOU tumor, circadian DNA synthesis.

Introduction. Cybernetics control mechanisms in biology and human physiology are based on *feedbacks* and *forwards* loops related with interaction between endocrine target glands via imaginary axes, however, without any consideration of the temporal structure (*chronome*). A temporal ex vivo interaction between cephalo-endocrine-immune networks: a modulator (pineal gland) interacts between the actor (pituitary) and reactor (adrenal gland) from such interactions emerged as the feedsideward [1]. Such temporal mechanism is characterized as by inhibition, no effect and enhancement of a marker rhythms (hormones or neurotrasmitter) (Figure 1). Each rhythmic component can alternated its role from reactor to modulator or actor. This mechanism [1] has been confirmed and extended to different chronomics levels from health to cancer disease, [2] might act as fourth interacting component or circadian disrupter affecting intermodulating integrity of biological spectrum of rhythms [3, 4].

Mazzoccoli et al. [5] reported the possible role of *feedsideward* on the cephalo-endocrine-immune chronome of lung cancer patients where melatonin is involved. This hormone is rhythmically produced by influence of light-dark cycles, as well as, heliomagnetic forces that might influence the incidence of cancer and tumor growth. It has been claimed that changes on shift works might be involved as carcinogenic factor in the incidence of some human cancers [6], however, experimental evidence had shown that "scrambling" the rhythms reduce the carcinogenesis process [7]. A classical study by Blask et al [8] demonstrated the anti-mitotic in vitro activity upon human breast cancer cells that were kept in culture (in darkness). Anti-hapoptotic and anti-aging actions of melatonin contrasted with the inhibition of some experimental mammary tumors, however, had shown any effect or enhancement in leukemia L1210 in mice. Cancer circadian

disruption (CCC) seems to be involved in the biologic time structure affecting the interactions of neuro-endocrine-immune *feedsidewards*, however, experimental and clinical evidences need to be explored.

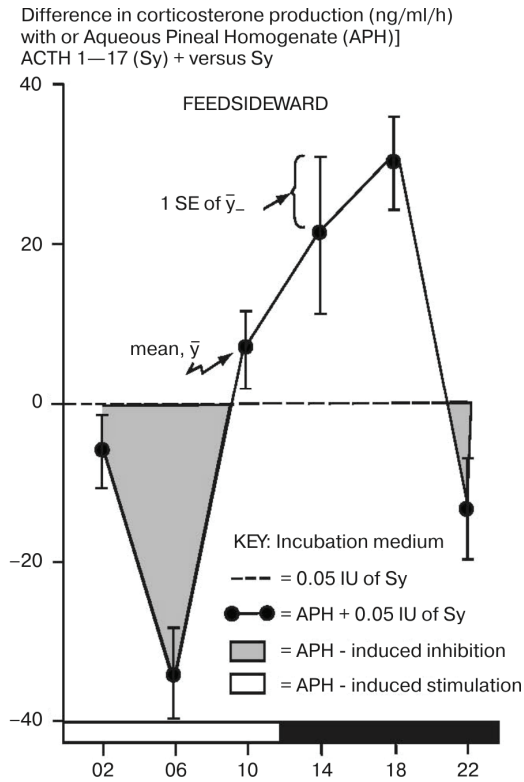

Fig. 1. Chronomodulation by aqueous pineal homogenate (APH) of ACTH 1—17 (Sy) effect upon adrenocortex:

This figure shows the feedsideward phenomenon gauged by corticosterone produced by quartered mice adrenal glands were incubated aqueous mouse pineal homogenated alone or with additions of 0.05 IU ACTH 1—17. Three main effects were observed from Inhibition, no effect and Stimulation. (Data obtained from Salvador Sánchez de la Peña — Ph.D. dissertation Thesis)

Aim. Against to this background here is attempted to explore the possible role of a chronomic biomarker: melatonin could be involved as possible modulator of cancer growth.

Material and methods. It was explored the circadian melatonin in vitro effects on ^{3}H-thymidine (Thy) incorporation into DNA myeloma fox (mF) cells. Incorporation of ^{3}H-Thy was determined by scintillation counter on 5×10^5 mF cells/200 μl of culture medium incubated at 37 °C in 5% CO_2 with additions of 50 and 500 pg of Mel. In vivo studies: Mel actions upon a) Meth-A-Sarcoma tumor growth in female Balb/C mice and b) Louvain (LOU) tumor growth implanted into LOU rats of both genders. Eighty eight female Balb/C mice 14-15 were kept on 12 h of light (L) alternating with 12 h of darkness. Inoculation of 1×10^6 Meth-A-Sarcoma cells was implanted subcutaneously in each mouse. Similar protocol was applied on LOU of both genders by inoculation of 1×10^5 LOU tumor cells, implanted subcutaneously in each rat. In both studies, five days after mice received a subcutaneous (SC) daily injection of 1 mg of Mel/kg or placebo

during 7 days at 6 circadian time points: 2, 6, 10, 14, 18 and 22 Hours After Light Onset (HALO). Eight groups of mice received daily injection SC at 10 and 18 HALO of diluent, ACTH, Corticosterone and melatonin alone or with additions of IL-2. Tumor was measured every day.

Results. Significant reduction of DNA synthesis of mF cells at 14 h after ^{3}H-Thy addition was observed with 50 and 500 pg of melatonin was administrated. Higher melatonin dose reduced DNA synthesis at 2, 6, 14 and 22 h after ^{3}H-Thy and kept circadian overall DNA synthesis ($p < 0.005$). Figure 2 shows this *chronomodulation* action of melatonin on DNA synthesis of myeloma fox cells. Circadian disruption of DNA synthesis was induced with lower melatonin doses (not shown).

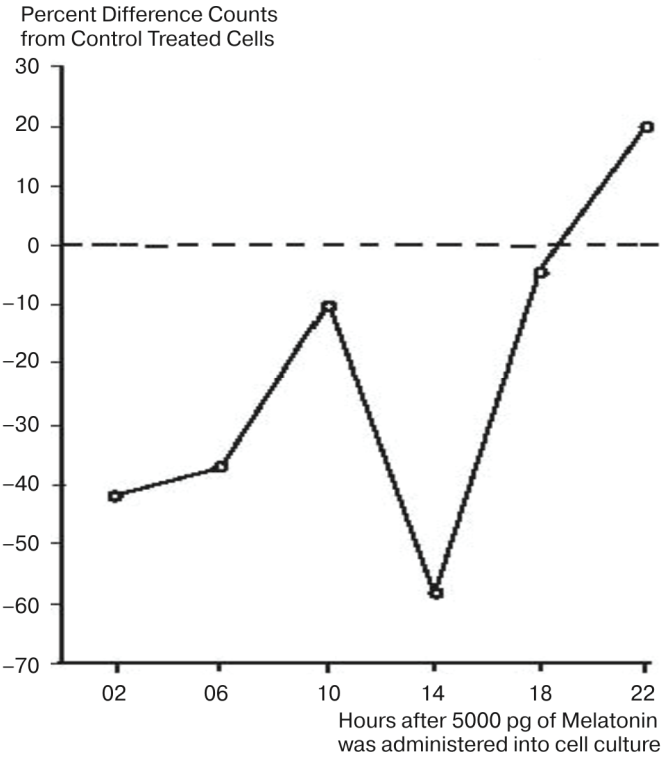

Fig. 2. In vitro Chronomodulation of M Fox Cell Growth by Melatonin.
The figure shows this *chronomodulation* action of melatonin on DNA synthesis of myeloma fox cells.

In vivo **results.** *First study.* Melatonin induced *chronomodulation* of LOU tumor growth in male rats and circadian disruption in the case of female treated rats. Figure 3 summarizes these effects, by comparison of tumor growth differences between the mean value of diluents treated rats shown as horizontal line equal to "0" and the original single values of tumor growth (mm 2 × 10) form, calculated at each of the six circadian times when melatonin was administered in each rat. In this manner two temporal series are shown i) On red color is related to the alternated changes from inhibition to enhancement of LOU tumor growth in female rats and ii) On black similar circadian *chronomodulation* of LOU tumor growth is observed in male rats. This Figure illustrates that during the beginning of light span a 20% of enhancement, as well as the 10% at the end of

activity span (darkness) of tumor growth is observed in male rats. Opposite action is observed in the growth of LOU tumor in the female rats. However, decrease of tumor growth is observed on both genders when it is administered at the end of light span (10 HALO). During the middle of dark span or activity, melatonin enhanced the tumor growth in the female rats and the opposite effect at 22 HALO.

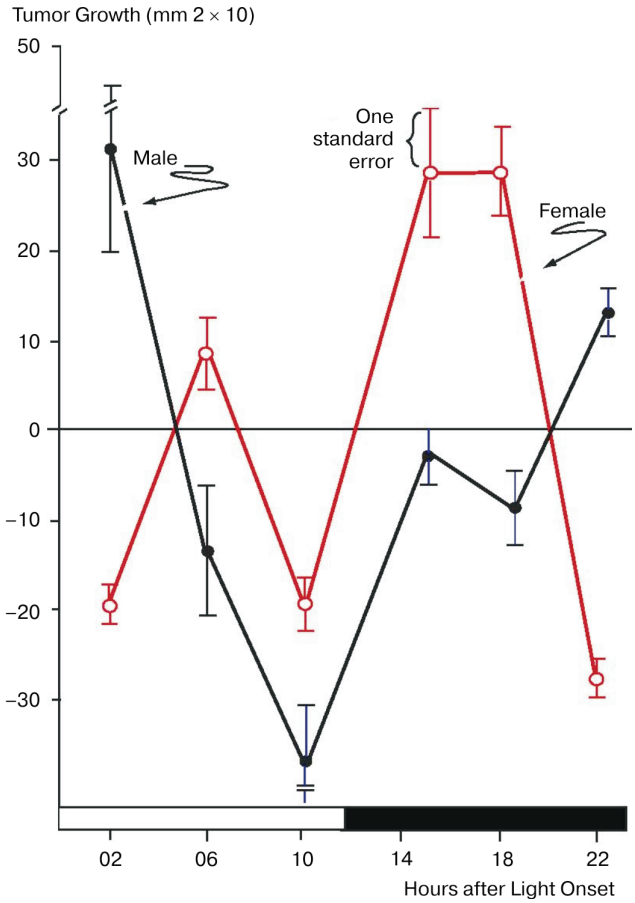

Fig. 3. Melatonin induced chronomodulation in LOU tumor growth (male rats) and circadian disruption of tumor growth in female rats:

This figure shows the *feedsideward* phenomenon of the immunocytoma growth induced by melatonin in LOU female and male rats, in this gender characterized by a) stimulation; b) inhibition and c) not effect and contrasting in female rat with alternation inhibition and stimulation of tumor growth in relation of time of melatonin intraperitoneally administration

Second study. Rather of selected at least 5 circadian points, it was selected only two circadian times based upon closed peak hormonal secretions of corticosterone (end of resting span) and melatonin (middle of mouse activity), as time of hormonal administration of ACTH, corticosterone an melatonin with or without addition of 4,500 IU of recombinant human (rh) IL-2 in female Balb/C mice previously inoculated with 1×10^6 viable Meth-A-sarcoma cells five days before at selected circadian time points: 10 and 18 HALO. In such manner that 2 diluent treatment groups and 14 subgroups received at 10 and 18 HALO: a) two subgroups with rhIL-2 alone; b) six subgroups with additions of 0.04 IU of ACTH [2] 1 mg of corticosterone [2] and c) 1 mg of melatonin/kg body weight [2] and six more subgroups of mice received only hormones alone

at 10 and 18 HALO. After tumor appearance was detected the surface was measured daily during 15 consecutive days. Data from tumor growth corresponding each of the two circadian times was expressed as percent of placebo or diluent treatments. In figure 4 a horizontal axis expressed the original values of each treatment as percent of tumor surface of mice treated with diluent (= 100%) at two circadian time points (10 and 18 HALO); seven vertical bars delay of tumor growth at 10 HALO due to rhIL2 and hormones (ACTH, corticosterone and melatonin) in relation to placebo treated mice or those treated at 18 HALO as is shown by seven red vertical bars.

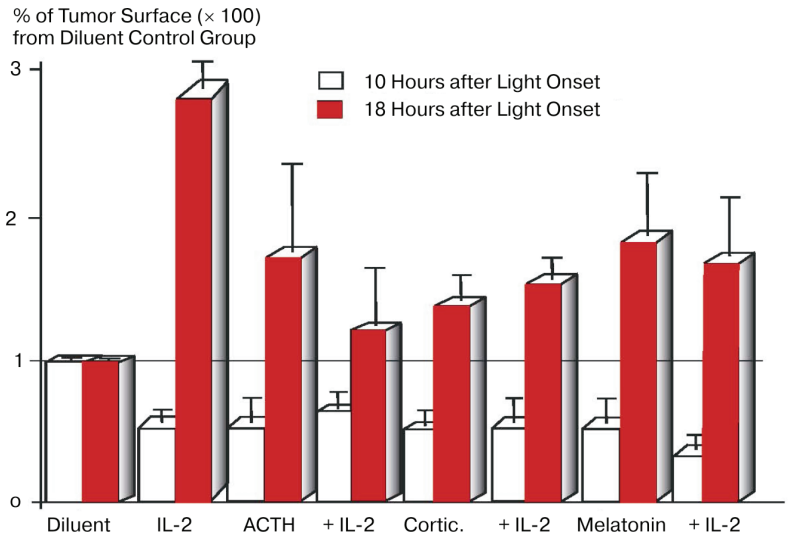

Fig. 4. Effect of various substances and their combinations on tumor growth depending on the circadian time

Eighty female Balb/C mice, 15 weeks of age were housed in a room with 12 hours of light (L) alternating with 12 h of darkness (D) with deionized water and food available at all times, for more than 3 weeks in two rooms which L-onset was staggered by 8 h. Meth-A-sarcoma cells were harvested at 02 HALO from peritoneal cavity of two mice. Cells were washed with 199 medium enriched with antibodies. After checking the viability of the cells 1x106 were inoculated subcutaneously (sc) into each mouse at 10 and 18 HALO. Five days after tumor inoculation (TI), 8 groups of mice were treated at the same times of TI with diluent (4—5 mice/group): 4,500 IU of human recombinant IL-2/mouse or combined with 0.04 IU of ACTH; 10 ug of corticosterone; or 1 mg of melatonin/kg of bw. Three more groups of mice were assigned to the treatment of each hormone alone. All treatments were given sc at the same 7 consecutive days. Tumor growth was determinated by daily surface measurements from each mouse. The data here presented was determinated 15 days after TI. Data from each treatment is expressed as percent of diluent treated mice. Each plotted bar represents the mean of 5—7 determinations

A higher enhancement of tumor growth is observed by rhIL-2 administrated at this circadian time. In contrast melatonin as well as, ACTH and corticosterone seems to decrease in part the enhancement of tumor growth, however all treatments at the middle of mice activity acted as accelerators of tumor growth. ACTH alone slightly stimulated tumor growth. In similar manner diminished IL-2 action upon tumor growth. This steroid administered alone, at this circadian stage has minor tumor growth stimulation. Administration of melatonin diminished IL-2 associated tumor growth stimulation and like ACTH and corticosterone had moderated effect upon tumor growth when is given lone. When all these treatments were administered during the mice resting activity (10 HALO) a clear inhibition of tumor growth was documented, as is shown on all white bars, as compared with the diluent treatmet (horizontal axis). IL-2 or ACTH halved tumor size, IL-2 with ACTH slightly diminished tumor growth relative to diluent. Corticosterone alone had

inhibitory effects equal to IL-2 alone, while giving them together resulted in a further reduction in tumor growth. Melatonin alone quartered tumor growth rate and melatonin plus IL-2 had identical anti-tumor effects. By comparing raw differences (not shown) between IL-2, melatonin and melatonin plus IL-2 a significant ($F = 2.6$; $P = 0.05$) treatment effect was observed by ANOVA. This intriguing results are part of an interacting rhythmic neuroimmune network via a *feedsidewards* mechanism.

Discussion. *Chronomodulatory* ex vivo actions have been demonstrated by administration of melatonin alone or with recombinant human (rH) IL-2 upon adrenal corticosterone (B) production [1]. Figures 5 and 6 summarized such *feedsidewards* upon adrenal glands or isolated cells, respectively. In figure 5 groups of female CD2F1 female mice were standardized for several weeks with 12 h of light alternating with 12 h of darkness. Groups of mice were euthanized at 6 different circadian time points, where adrenal glands were obtained, quartered and incubated during three hours with Krebs ringer buffer alone or with 10^{-7} M of melatonin. This indolamine induced two important effects: inhibition (shown by the light shadow) and stimulation (shown by dark shadow) of B production. Maximal inhibition is observed at 02 HALO contrasting with maximal stimulation at the beginning of activity span (14 HALO).

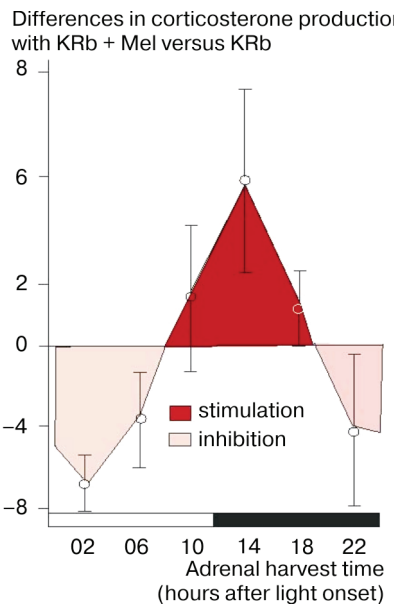

Fig. 5. Rhythmic modulator action of melatonin (Mel) upon adrenal corticosterone production.

Adrenals removed from female CD2F1 mice, ateach of 6 circadian times were bisected and incubated during three hours with KRb, with or without Mel. Mel inhibited at 02 HALO and stimulated at 14 HALO. Each plloted value represents 8—9 determinations

Figure 6 describes the *chronomodulatory* action of IL-2 upon the B production on isolated quartered adrenals, as well as on isolated cells from SHR-SP rats. As it was observed on Figure 5, IL-2 induced the same stimulation at 14 HALO as melatonin. However, in two different ex vivo preparations IL-2 induced inhibition of B produc-

tion at the end of resting and middle of activity spans. Such alternated effects observed during the circadian adrenal cycle by this lymphokine closed the loop between neuroendocrine and immune networks.

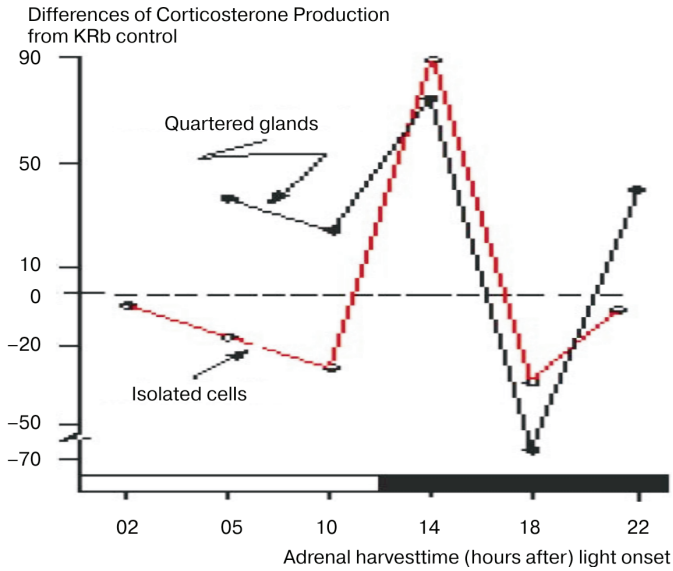

Fig. 6. *Chronomodulatory* action of recombinant human (rH) interleukin 2 (Il-2) upon corticosterone production from quavered adrenals or Isolates cells from male 5-HR rates.

Adrenals cells were obtained by collagenase treatment. Tissues or cells were incubated for 60 minutes at 37 °C with Krebs Ringer buffer (KRb– -) or with 2.0 μM of rH IL-2 (Cetus Co.) Each plotted value represents mean value of 5—7 determinations per circadian timepoint. Data are expressed as percent of KRb Incubated tissue or cells

It has been reported that shiftwork plays a role in the process of as carcinogenicity [6]. However, experimental induction of shifting the light-dark regimen on aging studies in mice and rats "scrambled" the rhythms prolonging life [7, 9—12]. The "circadian disruption" concept is simply narrowed. This concept must be extended to circadian-infradian intermodulations of the time structure (*chronome*). The neuroendocrine-immune networks are interacting in such manner to respond to the external photic and non-photic synchronizers via *feedsidewards*, where melatonin plays important role acting as chronomodulator in some cases by decreasing or enhanced tumor appearance [13] or inhibiting or accelarated tumor growth [1], depending of the integrity of mammalian *chronome*. Thus, melatonin might be involved with carcinogenetic and carcinostatic process. By focusing on simple control mechanisms without consideration of temporal structure the explanation of such controversy will remain without any clear understanding. Thus, the *feedsidewards* might be taken in consideration, where its integrity of circadian and infradian rhythmic intermodulation of rhythmic signals, such as melatonin upon neuroendocrine-immnune networks chronomodulates cancer tumor growth. Results presented here supported experimental confirmation of how melatonin has been involved at the cellular, tissue and entire organism affecting the *chronome* of tumor growth by keeping its circadian rhythmicity or inducing circadian disruption. Altered neuroendocrine and immune *chronomes* have been demonstrated in lung cancer patients [5]. Authors implicated that melatonin, in these patients, may regulate the response of

thyroid to hypothalamic-pituitary axis stimulation, playing a modulatory action on this network via *feedsideward* manner [5, 14], as has been demonstrated where the pineal gland and the hypothalamic-pituitary-adrenal networks [1].

A second neuroendocrine network constituted by pineal-hypothalamic-pituitary-thyroid [5] has been implicated now in patients with lung cancer by Mazzoccoli's Clinical research group and consider in some respect the feedsideward mechanisms. Considering the interactions in the control of body temperature as important biological marker [14].

Conclusions. Here the presence of a chronomodulating actions of melatonin via *feedsideawards* mechanism *in vitro* as well as, *in vivo*, in two experimental models was presented. Intriguing process of how this takes place may due to an interacting pineal pituitary-adrenal networks [1]. *In vivo* studies confirmed the role of melatonin in the study on Meth-A-sarcoma in mice and in LOU tumor growth. Melatonin disrupted circadian time structure of *in vivo* tumor growth on a *feedsideawards* manner in the case of the immunocytoma growth in female inoculated rats. Low doses disrupted the circadian DNA synthesis of mF-cells. Drug delivery systems must be taken in consideration the role of melatonin *feedsideawards* involvement during chronomodulated therapy of cancer patients.

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МЕЛАТОНИН: FEEDSIDEWARD МЕХАНИЗМ КАК СОВОКУПНОСТЬ ВСЕХ ВЗАИМНЫХ РЕГУЛЯТОРНЫХ СВЯЗЕЙ ГОРМОНА ЭПИФИЗА В ПРОЦЕССАХ РОСТА ОПУХОЛИ

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В проведенном исследовании показано наличие хрономодулирующего действия мелатонина, реализуемого посредством механизма feedsideawards, т.е. совокупности всех взаимных регуляторных связей мелатонина, как in vitro, так и in vivo, на двух экспериментальных моделях. Сложные, до сих пор достоверно не обоснованные, процессы могут быть обусловлены взаимодействующими связями с эпифиз-гипоталламо-надпочечниковой системой. В исследовании in vivo была изучена и подтверждена роль мелатонина в развитии саркомы экспериментальной линии Meth A в группе подопытных мышей и опухоли Louvain. При моделировании роста опухоли нейроэндокринной системы на подопытных самках крыс было выявлено деструктивное влияние мелатонина на циркадианный ритм роста опухоли, реализуемое по принципу feedsideawards. Малые дозы мелатонина нарушают синхронизированный с циркадианным ритмом синтез ДНК в клетках mF миеломы лисы. В лечении онкологических пациентов, соответствующем требованиям хрономедицины, должна учитываться роль совокупности всех взаимных регуляторных связей мелатонина, формирующих механизм feedsideawards, при разработке системы распределения лекарственных препаратов в организме пациентов.

Ключевые слова: мелатонин, хрономодулирующее действие, эпифиз-гипоталламо-надпочечниковая система, опухоль Louvain, опухоли нейроэндокринной системы, циркадианный ритм синтеза ДНК.