Neuroendocrinology Letters Volume 38 No. 7 2017
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Melatonin with adenosine solubilized in water and stabilized with glycine for oncological treatment – technical preparation, effectivity and clinical findings

Giuseppe Di Bella [1], Luciano Gualano [2], Luigi Di Bella †

1 Di Bella Foundation, Via Guglielmo Marconi 51 Bologna, 40122 Italy
2 Private Laboratory of Physiology, Via Stefano Giovanni Marianini, Modena 41123, Italy

Correspondence to: Dr. Giuseppe Di Bella
Via Marconi 51 "Di Bella Foundation"
Post code 40122, Bologna, Italy.
TEL: +39 051 239662; FAX: +39 051 230369; E-MAIL: posta@giuseppedibella.it

Submitted: 2017-09-05 *Accepted: 2017-11-23* *Published online: 2017-00-00*

Key words: **melatonin; adenosine; glycine; hydrogen bond; apoptosis; Di Bella Method**

Neuroendocrinol Lett 2017; **38**(7):101–110 **PMID:** ----- NEL380717AXX

Abstract

Melatonin has shown the potential to inhibit growth of different tumors, both in vitro and in vivo. There is clear evidence that the administration of melatonin alone or in combination with chemo and radiotherapy in cancer patients with advanced solid tumors has been associated with improved outcomes of tumor regression and survival. Moreover, chemotherapy has been shown to be better tolerated in patients treated with melatonin. However, there are different ways of preparation and administration of melatonin to the patient. This review article aims to offer the insight into the preparation, biological features and clinical findings in its use in cancer patients.

Melatonin (MLT) can only be solubilized in water at 40–45 °C; at other temperatures it can only be solubilized in alcohol. It is absorbed in the human body complexed with adenosine by a hydrogen bond. It acts on two common denominators: proliferation and differentiation; in addition to anticancer homeostasis, MLT has a documented antidegenerative and immunomodulatory role. It also plays an important role in limiting oxidative stress, affecting blood and bone marrow constituent ratio, leukocyte formula regulation, hemoglobin synthesis, platelet genesis, aggregation and in erythrocyte resistance. Despite of all these important roles, most well-known features are probably the least important ones, such as sleep and wakefulness regulation and its effect on jet lag.

In the preparation formulated by Prof. Di Bella, melatonin with adenosine at a ratio of 1:4, stabilized with 30% of glycine (MLT-DBM), has been used since 1994 in many patients with various indications and positive therapeutic responses and a total absence of toxicity. This method can be a good alternative to commercially produced preparations, as it was scientifically proved and published worldwide at conferences and in various medical journals.

REVIEW ARTICLE

Abbreviations:

AMP - Adenosine Monophosphate
APUD - Uptake and Decarboxylation
AR - Androgen Receptor
ATRA - All Trans Retinoic Acid
CASPASE - Protease Enzyme
CX 32 - Gap junction protein gene
DBM - Di Bella Method
DNES - Diffuse NeuroEndocrine System
EGF - Epidermal Growth Factor
EGFR - Epidermal Growth Factor Receptor
ER - Estrogen Receptor
FGF - Fibroblastic Growth Factor
GF - Growth Factor
GH - Growth Hormone
GHR - Growth Hormone Receptor
GMP - Guanosine Monophosphate
5-HT - Serotonin
HIOMT - Hydroxyindole-O-methyltransferase
IFN - Interferon
IL - Interleukin
MLT - Melatonin
MT1,MT2 - Melatonin Receptors
NAT - N-acetyltransferase
NGF - Nerve Growth Factor1
PKA - Protein Kinase A
RAR,ROR,RXR,RZR - Retinoid Receptors
RAS - Rat Sarcoma Protein
SST - Somatostatin
SSTR - Somatostatin Receptor
TGF - Transforming Growth Factor
VEGF - Vascular Endothelial Growth Factor
WV - Weight, Volume

INTRODUCTION

Melatonin with adenosine at a ratio of 1:4, stabilized with around 30% of glycine, has been used since 1994 in the DBM, in pharmaceutical freeze-dried form. In the pharmaceutical preparation according to the Di Bella method, melatonin is conjugated to adenosine by freeze-drying, in order to ensure greater bioavailability of the medication. Melatonin is able to form a complex with adenosine, probably the π type, due to orbital overlap of the aromatic systems and of the electronic double bonds of the nitrogen atoms. The complex is then stabilized by glycine, which, because of the fairly low pKa, contributes to the formation of hydrogen bridges. The formation of the complex involves a considerable variation with respect to the characteristics of the individual components: the complex is fully soluble in water, at concentrations at which adenosine and melatonin alone would precipitate or would not even dissolve.

PRODUCTION AND ADMINISTRATION OF THE MLT

Although it is still a galenic preparation, the method of production is regulated to ensure maximum product quality. Good preparation standards are applied to the processing stages and the end product is analyzed by qualitative and quantitative determination of the active ingredients.

The freeze-drying of the water solution of melatonin-adenosine-glycine is regulated by a set-up method. The freeze-dried form is extremely hydrosoluble and can be prepared in vial form for oral use, intramuscular or intravenous injection. Freeze-drying process effectively dehydrates various substances, in a way that the end product retains its particular characteristics and subsequently sterile distilled water for injectable preparations is added, to restore the specific properties of the original solution. The freeze-dried product can also be used in oral vials, in which the water is added at the time of administration.

Melatonin itself is very difficult to dissolve in water, with satisfactory solubility only at 40–45 °C. After lengthy testing, adenosine was found to be the most suitable molecule to easily dissolve melatonin in water. In particular, the ideal ratio was found to be four moles of adenosine (267.24 g) to one mole of melatonin (232.28 g).

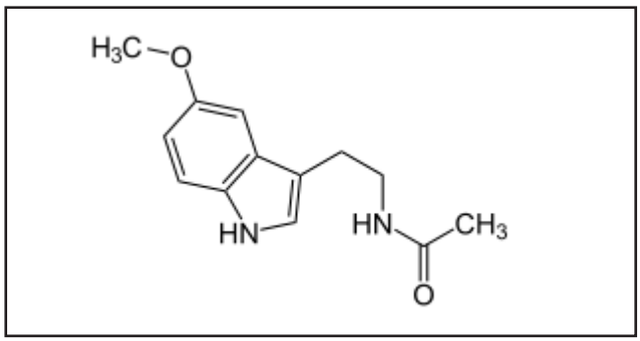

Fig. 1. Melatonin (MLT), C13H16N2O2 . Synonyms: N-acetyl-5-methoxytryptamine; N[2-(5- Methoxy-1H-indol-3-yl)ethyl] acetamide (Di Bella G *et al.* 2013).

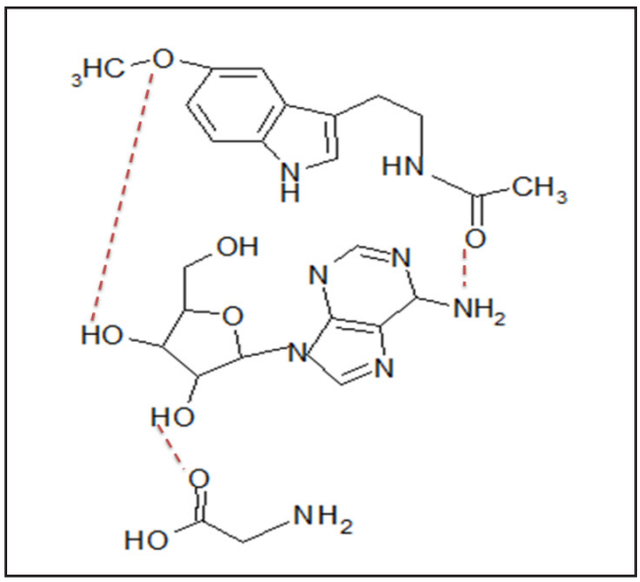

Fig. 2. Melatonin plus adenosine and glycine. Hydrosoluble Melatonin according to Prof Luigi Di Bella's formulation.

The preparation of melatonin with a high degree of purity consists of the following steps:

a. reacting potassium phthalimide with dibromopropane to obtain 3-bromopropilphthalimide;
b. reacting 3-bromopropilphthalimide with sodium acetoacetic ester to obtain ethyl-2-acetyl phthalimidopentanoate;
c. reacting ethyl 2-acetyl phthalimidopentanoate with diazo-p-anisidine to obtain 2-carboxyethyl-3-(2-phthalimidoethyl)-5-methoxy-indole;
d. reacting 2-carboxyehyl-3-(2phthalimidoethyl)-5-methoxy-indole with sodium hydroxide and then with sulphuric acid to obtain impure 5-methoxytriptamine;
e. reacting impure 5-methoxytriptamine with hexamethyldisilazane to obtain a mixture of mono- and di-substituted derivatives and hydrolyzed with aqueous methanol to obtain pure 5-methoxytriptamine;
f. reacting pure 5-methoxytriptamine with acetic anhydride to obtain impure melatonin and purifying this melatonin by means of chromatography on silica gel and first eluting with methylene chloride followed by eluting with methylene chloride and acetone to obtain a solution, concentrating this methylene chloride and acetone solution to obtain a solid, and recrystallizing this solution to obtain melatonin.

The method according to step *d* involves refluxing at a 135 °C for 2 hours until complete solution is obtained, then adding a p 20% (W/V) H_2SO_4 solution and further refluxing for four hours. After refluxing with 20% sulphuric acid, the solution is cooled to let the phthalic acid precipitate and the phthalic acid is filtered off. After the phthalic acid is filtered off, sodium hydroxide is added and impure 5 methoxytriptamine is extracted with methylene dichloride. After refluxing for 12/14 hours, the impure 5-methoxytriptamine is treated with hexamethyl disilazane, to obtain mono and di-silyl substitution products, then the solution is distilled under normal pressure so as to recover excess hexamethyl-disilazane and the silyl substitution products are hydrolyzed with aqueous methanol in order to obtain essentially pure 5 methoxytriptamine (Di Bella L *et al.* 1997).

Glycine in the vials is present both as a freeze-drying co-adjuvant and as an agent making the pharmaceutical form isotonic; as it is not conveyed by intestinal adenosine, being bound to exogenous adenosine with a hydrogen bond is even more important for injectable melatonin. The vials must respect the technology for sterile-apyrogenic preparations. Parenteral route is usually preferred when difficulties with the absorption of melatonin by the digestive tract or inability of its ingestion are present and when it is necessary to administer high doses. Just before use, vials of freeze-dried MLT should be diluted in 10 ml of distilled sterile water for injectable preparations. It is not necessary to use saline solution as the presence of glycine makes it isotonic. The quantity obtained can be injected in a single administration, but very slowly because adenosine is a vasodilator and could lead to a drop in blood pressure. It is therefore advisable to test the patient's reactivity by using 1 ml of solution. The solution can be injected intramuscularly or intravenously.

BIOLOGICAL FEATURES OF MLT AND ITS ANTICANCER ACTIVITY

Numerous studies have described the in vitro effects of MLT on the proliferation of tumor cell lines and on their apoptosis. The dynamics involved in the division of normal cells and tumor cells depend on and are coordinated by a hierarchy of circadian timekeepers. The bio-activities of MLT are either available or not in *vivo* in a coordinated way, in specific circadian phases (Bartsch *et al.* 1997, 1999). The authors conclude that the numerous data on the influence of MLT on tumor biology in vitro indicate that the circadian state of administration of MLT to cancer patients also determines its anticancer activity. It has been demonstrated that MLT has a dose-dependent, antioxidant and experimentally reproducible effect, with significant implications in the prevention and treatment of tumors for the protection of nuclear and mitochondrial DNA from the potentially neoplastic oxidative stress (Kojima *et al.* 1997; Reiter *et al.* 2000).

The ability of MLT to protect DNA from damage caused by carcinogenic chemical substances is also a decisive factor. These concepts were applied in clinical practice by Prof. Di Bella, wherein the biochemistry of MLT or of the other pineal indoles, the presence of taurin and of many peptides, the innervation or the functional circadian or seasonal cycles, or the correlation with the hypophysis or other endocrine glands or releasing factors can fully clarify the antiblastic mechanisms of MLT action. In these reactions, which lead to the production of both NO and polyamines, MLT can play a fundamental role. In the book "Cancro, siamo sulla strada giusta" (Di Bella L 1998), Prof. Di Bella put forward a new interpretation of this complex problem, the ability of MLT to interact with the tumor biology, in relation to the central dogma of biology. According to the current concepts of molecular biology, a fundamental role is played by the preexistence of information for every amino acid sequence, the element of protein synthesis forming the basis for physiological and neoplastic life in its essential expressions of morpho-functional proliferation and differentiation. Di Bella, in fact, identified a primary role of MLT in the ubiquitous arrangement of the phosphoric esters of AMP, ADP, and ATP. This concept is shared by the school of thought headed by Goldberger (Blasi *et al.* 1971; Meyers *et al.* 1975), who also admits the possibility of self-assembly, and that the protein can spontaneously re-acquire its three-dimensional structure with full biological activity. It could be the same or another

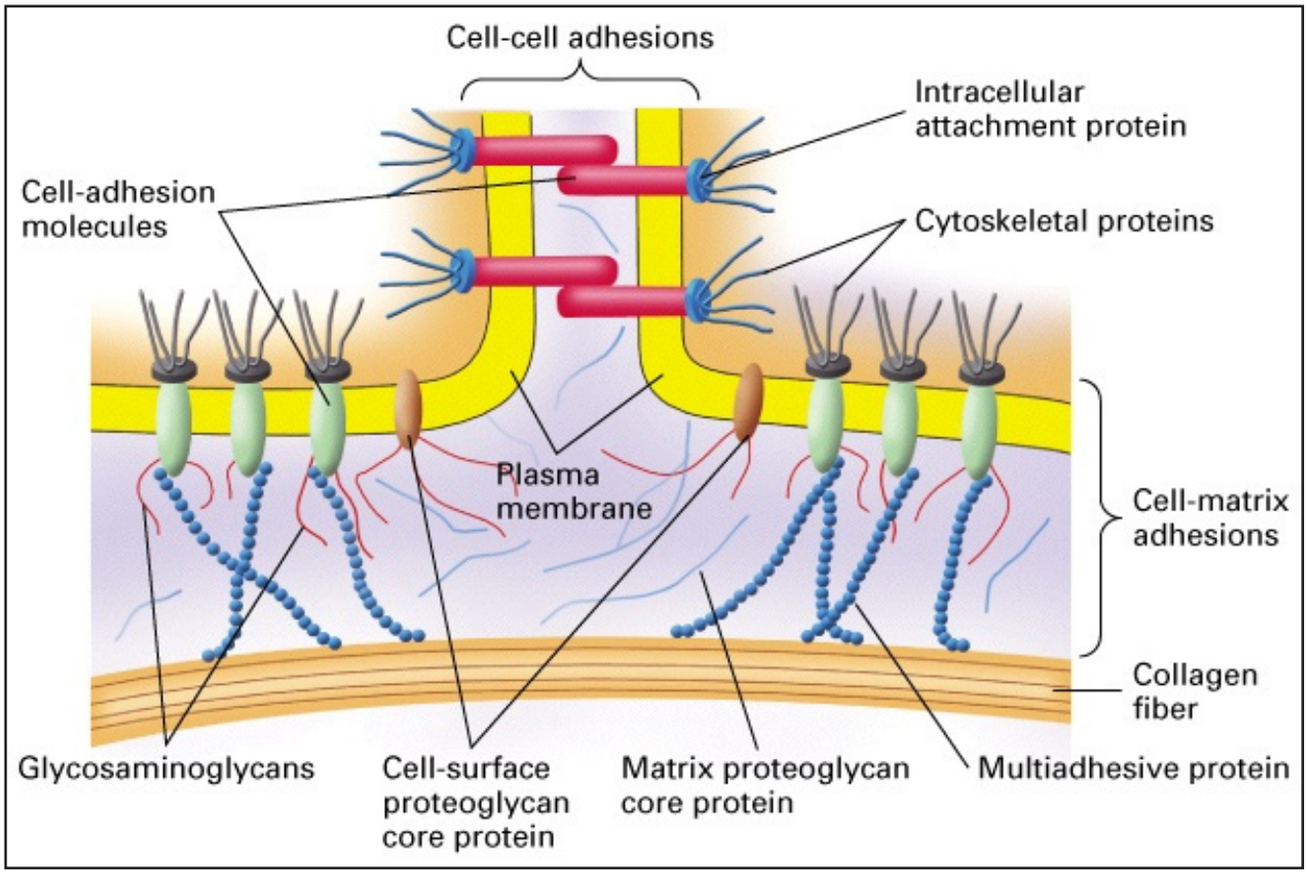

Fig. 3. Cell-cell adhesion. Melatonin induces protein CX-32 of the junction spaces and increases the polymerization of tubulin with an increase of microtubules in the cells (Alberts *et al.* 2002).

protein which influences the intermolecular reactions. Some proteins act as molecular chaperones and by hydrolyzing ATP activate the deployment of inert protein structures without this reaction. The mechanism of action was explained by Ellis (1996), who defined the chaperonins as sequestrants containing the individual protein structures folded in the Anfinsen Cage. According to Prof. Di Bella, in tumor biology the action of the chaperonins takes place mainly through the hydrolysis of ATP, ADP, and AMP, their binding with adenosine, or in theie hydrogen bond with MLT. In the last decade, the attention of many researchers was focused on MLT, formerly considered merely an epiphyseal secretion, while it is now believed to be a component of the Diffuse Neuro-Endocrine System (DNES), also known as Amine Precursor Uptake and Decarboxylation (APUD) present in many more or less ubiquitous cellular aggregates, also able to produce MLT (Kvetnoy *et al.* 1986).

Platelets can be considered omnipresent, multifactorial and itinerant elements of a changeable and ubiquitous APUD system. Platelets sometimes behave like a melatonergic and dopaminergic, serotonergic and adrenergic neuron, according to the various local conditions and attracting nature of the nuclei. Platelets can absorb and store 5-TH; they can also synthesize MLT since they too are provided with 5-TH-decarboxylase. A large amount of pharmacological data demonstrated considerable functional affinity between the platelets and neurons of the serotonergic system. This function of the platelets, which release their stores of 5-HT and expel material from their granules when they are activated by appropriate signals, was considered very similar to the release of neurotransmitters by the central neurons. The release of biologically active molecules by the platelets is similar to that of the serotonergic and adrenergic central neurons (Kvetnoy *et al.* 1986; Di Bella L 1998).

Variable concentrations of MLT have been found in the following locations: retina, Harder glands (tear glands), intestinal mucosa, cerebellum, epithelium of the airways, liver, kidneys, adrenal glands, thymus, thyroid, pancreas, ovaries, testicles, carotid sinus, placenta, endometrium, mast cells, natural killer cells, leukocytes, eospinophils, endothelial cells and also in platelets and megakaryocytes, as shown by the research carried out by Prof. Di Bella on the platelet-megakaryocyte-MLT interaction. This generalized diffusion shows that MLT has a unique role among the components of the DNES/APUD system and is an essential component of the response and control of the body's anticancer protec-

tion system, acting on all organs. MLT can be considered extrapineal, as a key molecule of the paracrine system for the local coordination of the intercellular reactions, an irreplaceable element in the prevention and treatment of tumors. The fact that many cells adjacent or close to the production sites of MLT have membrane receptors for MLT confirms the above findings. Kvetnoy *et al.* have studied and experimentally confirmed the direct participation and active role of MLT and DNES/APUD hormones on the etiopathogenesis and proliferation of tumors and on antiblastic therapy (Kvetnoy *et al.* 1994, 1997, 2002). An analysis of the physiological characteristics of many biologically active substances produced by the cells of the DNES/APUD system, such as melatonin, serotonin, gastrin, insulin, glucagon, somatosatin, etc., suggest an important function of these cells and hormones in tumor growth. A study of the role and significance of the DNES/APUD system, and above all of the melatonin-secreting extrapineal cells, in tumor pathogenesis provides a new interpretation of the endogenous mechanisms of the responses induced by tumors in various organs and tissues. Hyperplasia of the enterochromaffin cells that produce MLT, of the BETA pancreatic insulin secreting cells, of the somatostatin-producing D cells, and of the noepinephrine-producing adrenal NEP cells has been documented in the tumor onset and proliferation stages, while there is a significant decrease in the number of these cells in the more advanced or terminal stages of cancer. The experimental studies by Zabezhinskii *et al.* (1999) have shown the same behavior of these cellular aggregates in Lewis lung carcinoma in mice. Early-stage circumscribed tumors, with a modest degree of proliferation, only slightly differentiated, not

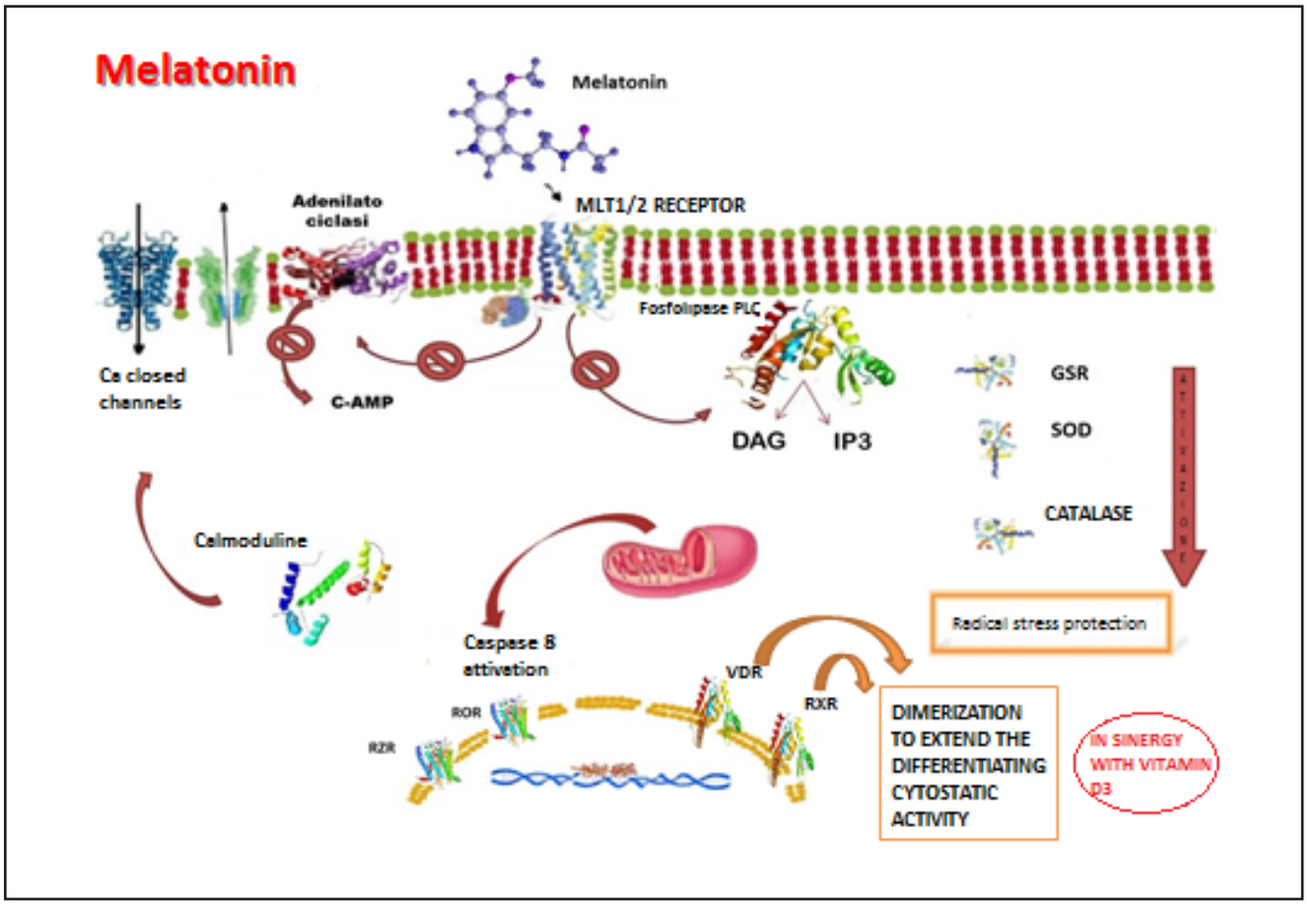

Fig. 4. 1) Melatonin negatively regulates phospholipase C and the second messengers IP3 and DAG. 2) Melatonin also activates the intrinsic mitochondrial pathway that leads to activation CASPASE 8 thus inducing apoptosis. 3) Melatonin is responsible for activation of a cytostatic pathway when in contact with the cytosolic protein Calmodulin; the MLT-Calmodulin bond reduces the levels of cytosolic Ca2+ and closes, at the level of the plasma membrane, the Ca2+ channels at their entry point. 4) At the level of the nuclear membrane, melatonin interacts with RZR (orphan) and ROR receptors, which can also be activated by the retinoids. The receptors RAR and RXR of the retinoids and VDR of vitamin D, ROR and RZR dimerize and thus activate, in an amplified way, the nuclear transcription factors through methylation reactions, silencing the sequences of the mutation genes with a cytostatic side effect. 5) The hydrosolubilization of Melatonin, increasing its bioavailability and ubiquitous diffusion, facilitates and reinforces the ability to bind the nuclear receptors RZR, ROR, transmembrane receptors (MT1 and MT2) with 7 transmembrane domains associated with G proteins which activate multiple signaling lines: – The bond with the MT1 receptor inhibits adenylate cyclase and consequently the second messenger (cAMP) and the reactions of phosphorylation of the protein PKA; – The bond with the MT2 receptor inhibits guanylate cyclase and the formation of GTP, and of the protein RAS (Di Bella G *et al.* 2013).

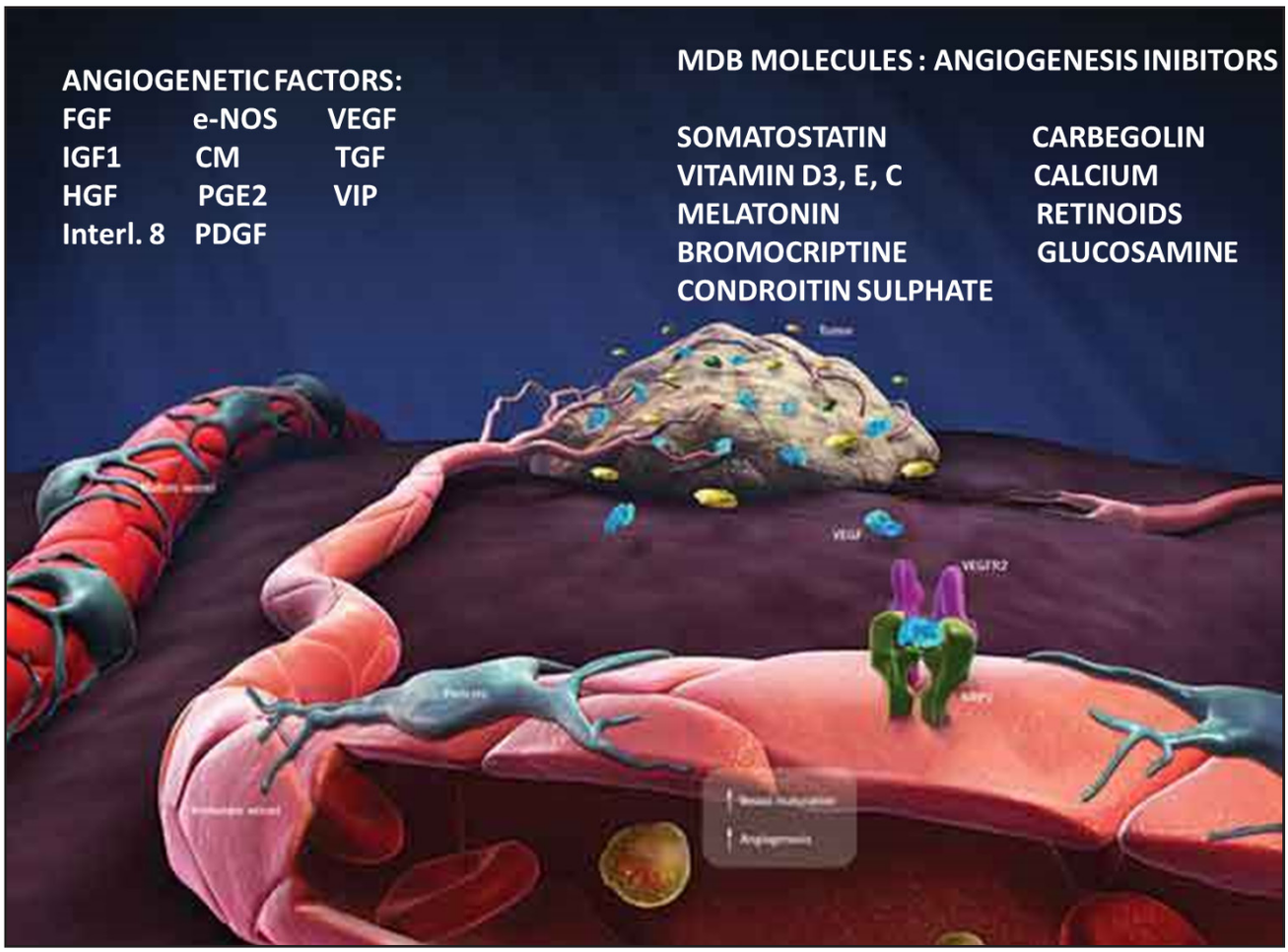

Fig. 5. The hydrosolubilization of Melatonin, increasing its bioavailability and ubiquitous diffusion, facilitates and reinforces the ability to bind the nuclear receptors RZR, ROR, transmembrane receptors (MT1 and MT2) with 7 transmembrane domains associated with G proteins which activate multiple signaling lines: – The bond with the MT1 receptor inhibits adenylate cyclase and consequently the second messenger (cAMP) and the reactions of phosphorylation of the protein PKA; – The bond with the MT2 receptor inhibits guanylate cyclase and the formation of GTP and of the protein RAS.

metastized, show less activity, demonstrated by the morpho-functional aspects and by hypoplasia of the intestinal enterochromaffin cells, including the histamine–producing ECL cells, the G cells of the stomach which produce gastrin, the pancreatic A cells, which secrete glucagon, the adrenal EP cells which secrete epinephrine. All these cell aggregates increase considerably both in number and activity as tumor proliferation, and invasion proceeds, reaching an evident degree of hypertrophy in the more advanced or terminal stages. In experimental studies on male Wistar rats with sarcoma-45, a significantly greater concentration of MLT was observed (seen by the bonding of its radiomarked isotopes 3H-MLT and 125 Iodo-MLT), with an evident homeostatic function of limiting tumor expansion in many vital organs such as the intestine, liver, respiratory epithelium, kidneys adrenal glands and pancreas. The absence of an increase in the melatonin concentration in these organs in healthy animals confirms that MLT is an anticancer homeostatic mechanism (Mediavilla *et al.* 1997; Di Bella L *et al.* 2015).

Melatonin use in cancer patients have started in 1974, when melatonin prepared according to Prof. Di Bella's formulation, in 25 mg bottles, at high doses of a thousand milligrams per day for 11 days was administered to the patient. At the request of the patient and family members with informed consent, and with the consent of the hospital management and the relative consultant, in view of the lack of therapeutic alternatives, a forty-year old patient, admitted to the general medical ward at the Maggiore-Pizzardi Hospital in Bologna, with chemo and radiotherapy-resistant lymphosarcoma after repeated cycles of chemo and radiotherapy, with bilateral laterocervical, axillary lymph node mediastinic, bilateral inguinal and splenic progression, was very slowly (over approx. 8 hours) and intravenously administered 1000 mg of melatonin for 11 days. During the course of each day, the patient was intravenously administered 4 saline drips of 500 ml, each containing ten 25 mg bottles of freeze-dried melatonin, lasting 2 hours, totaling 1000 mg per day. No other drug of any kind was administered in order to ascertain the effect

of the MLT without interference. A complete and stable objective response was observed and radiographicly documented, recorded in the patient's medical notes. The patient passed away in due to acute meningitis 15 years later. It was not possible to administer similar doses in other cases because the supplier (IFLO, Milan) stopped its production and because of the lack of hospital ward cooperation. Lymphoproliferative diseases are particularly sensitive to the action of DBM melatonin, as we have seen in many other published cases. The rapidity of the response in the case described above, the total absence of toxicity and the stability of the result suggest that significant progress in cancer treatment could be achieved, with particular efficacy in lymphoproliferative forms, as confirmed not only by this single case but also by the numerous reported cases of oral administration of the DBM (Di Bella & Fraschini 1997; Todisco *et al.* 2001, 2009; Di Bella G *et al.* 2012).

DISCUSSION AND CONCLUSIONS

The positive anticancer effect in terms of objective response, survival rates, performance status, tolerability and the absence of significant toxicity has been ascertained in thousands of patients, reported at national, European and international conferences, and published in around thirty scientific papers, documenting around eighty cases of a variety of types of tumor: sarcomas (Di Bella G *et al.* 2016), glioblastomas (Di Bella G *et al.* 2015), breast cancer (Di Bella G 2008, 2011; Di Bella G *et al.* 2013) , prostate cancer (Di Bella G *et al.* 2013) , neuroblastomas (Di Bella G *et al.* 2009), esophageal cancer (Di Bella G *et al.* 2009), non-small cell lung cancer (Norsa *et al.* 2006), cervicofacial tumors (Di Bella G *et al.* 2012), lymphoproliferative diseases (Todisco *et al.* 2001, 2009; Di Bella G *et al.* 2012).

Prof Luigi Di Bella defined the integration of MLT in his method containing immunomodulating, trophic, differentiating, and antiproliferative molecules, saying that any medical treatment not including Melatonin was unable to completely cure and stabilize a tumor, and that it represents a necessary condition albeit not sufficient. He summarized his therapeutic reasoning and biological method as: "being more essential than the impracticable and imaginary killing of all the tumoral elements, the achievement of all the conditions known, possible and not dangerous within certain limits, capable of hindering their development, as far as death also by apoptosis, especially through the interplay between numerous growth factors. The essential lies in activating all the inhibitors of the known growth factors at the right doses and at the right time. The DBM protocol was created in this setting, the setting of life, not that of intoxication and cell death, a *method that supports or enhances vital reactions*, without using statistical precision to find the most appropriate doses

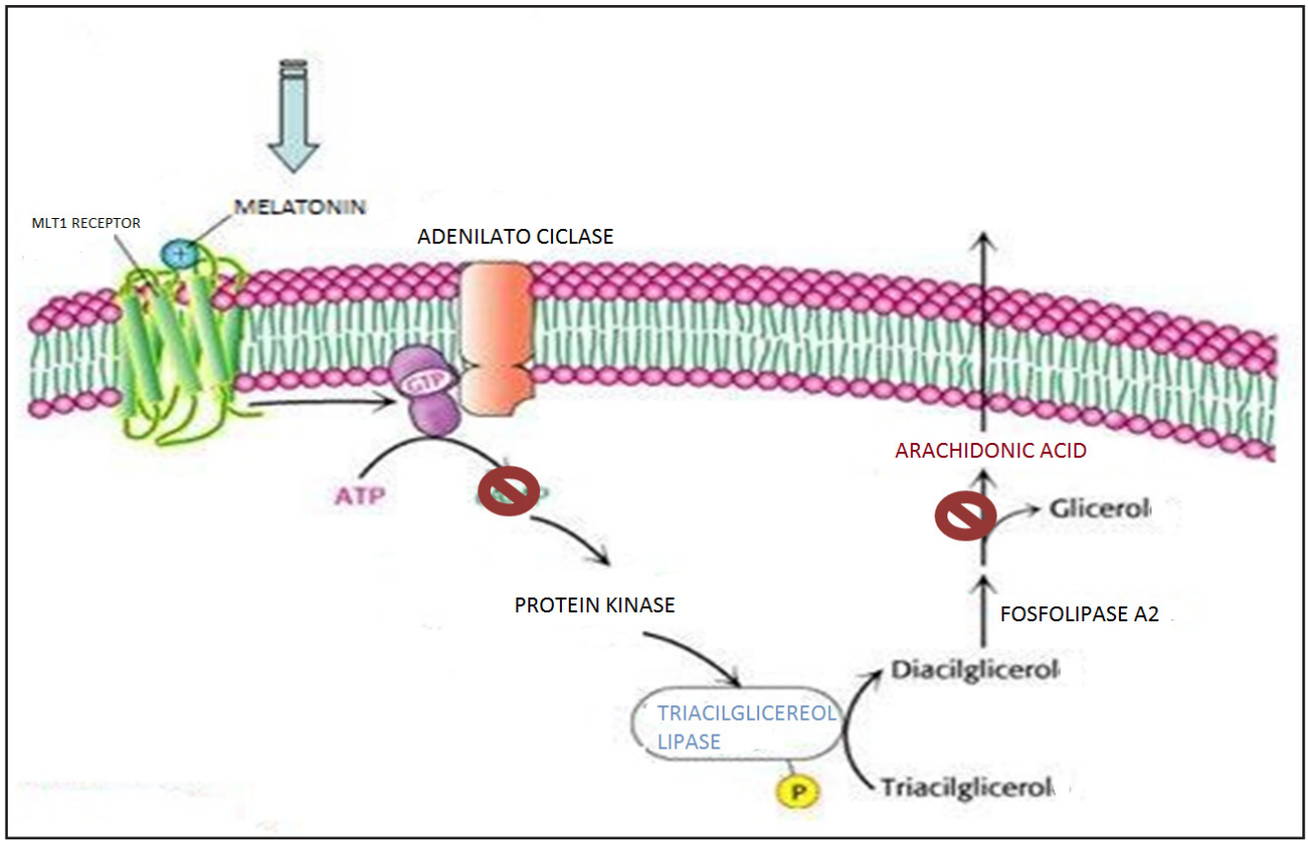

Fig. 6. Melatonin is able to inhibit the release of fatty acids from adipose tissues and the absorption of fatty acids by tumors.

to kill. A tumor is a deviation from normal life, so the reactions that have deviated must be returned to their normal status by enhancing those means that physiology considers to be essential for normal life"; and "there is, nor ever will there be, no cytotoxic chemotherapy (nor monotherapy) that can cure a solid tumor, only a method, a rational and biological multitherapy, a complex of synergic and factorially interactive substances, each with its own individual atoxic anticancer activity and sequentially or simultaneously acting centripetally together on the myriad of biological reactions involved in the tumoral life, leading gradually to normality of the vital reactions deviated by the tumor." (Di Bella & Rossi 1979; Di Bella L *et al.* 1980; Di Bella L 1997; Di Bella L & Gualano 2006; Di Bella G *et al.* 2013).

A review of the literature confirms the considerable functional versatility of MLT, which can in fact have both a direct and indirect anticancer effect, working in synergy with other differentiating, antiproliferative, immunomodulating and trophic molecules of the anticancer treatment formulated by Luigi Di Bella (Di Bella Method, DBM: Somatostatin, Retinoids solubilized in vitamin E, Ascorbic Acid, Vitamin D3, Prolactin inhibitors, Chondroitin sulfate). The interaction of MLT with the DBM molecules counters the multiple processes that characterize the tumor phenotype (induction, promotion, progression and/or dissemination, tumoral mutation). All these features suggest the use of these molecules in oncological diseases may be recommended.

REFERENCES

1 Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002). Molecular Biology of the Cell. 4th ed. New York: Garland Science.
2 Bartsch C, Bartsch H (1997). Significance of melatonin in malignant diseases. Wiener Klinische Wochenschrift. **109**(18): 722–729.
3 Bartsch C, Bartsch H (1999). Melatonin in cancer patients and in tumor-bearing animals. Adv Exp Med Biol. **467**: 247–264.
4 Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke D, *et al.* (2000). The pineal Gland and cancer. Neuroimmunoendocrine Mechanisms in Malignancy. Springer-Verlag Berlin Heidelberg,
5 Bartsch C, Bartsch H, Buchberger A, Stieglitz A, Effenberger-Klein A, Kruse-Jarres JD, *et al.* (1999). Serial transplants of DMBA-induced mammary tumors in Fischer rats as a model system for human breast cancer. VI. The role of different forms of tumor-associated stress for the regulation of pineal melatonin secretion. Oncology. **56**(2): 169–176.
6 Bartsch C, Kvetnoy I, Kvetnaia T, Bartsch H, Molotkov A, Franz H, *et al.* (1997). Nocturnal urinary 6-sulfatoxymelatonin and proliferating cell nuclear antigen immunopositive tumor cells show strong positive correlations in patients with gastrointestinal and lung cancer. J Pineal Res. **23**(2): 90–96.
7 Bizzarri M, Cucina A, Valente MG, Tagliaferri F, Borrelli V, Stipa F, *et al.* (2003). Melatonin and vitamin D3 increase TGF-beta1 release and induce growth inhibition in breast cancer cell cultures. J Surg Res. **110**(2): 332–337.
8 Blasi F, Barton RW, Kovach JS, Goldberger RF (1971). Interaction between the first enzyme for histidine biosynthesis and histidyl transfer ribonucleic acid. J Bacteriol. **106**(2): 508–513.
9 Blask DE, Sauer LA, Dauchy RT (2002). Melatonin as a chronobiotic/anticancer agent: biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr Top Med Chem. **2**(2): 113–132.
10 Chang WP, Lin CC (2017). Relationships of salivary cortisol and melatonin rhythms to sleep quality, emotion, and fatigue levels in patients with newly diagnosed lung cancer. Eur J Oncol Nurs. **29**: 79–84.
11 Codenotti S, Battistelli M, Burattini S, Salucci S, Falcieri E, Rezzani R, *et al.* (2015). Melatonin decreases cell proliferation, impairs myogenic differentiation and triggers apoptotic cell death in rhabdomyosarcoma cell lines. Oncol Rep. **34**(1): 279–287.
12 Cos S, Verduga R, Fernández-Viadero C, Crespo D (1996). Effects of melatonin on the proliferation and differentiation of human neuroblastoma cells in culture. Neurosci Lett. **216**(2): 113– 116.
13 Dauchy RT, Blask DE, Sauer LA, Davidson LK, Krause JA, Smith LC, *et al.* (2003). Physiologic melatonin concentration, omega-3 fatty acids, and conjugated linoleic acid inhibit fatty acid transport in rodent hind limb skeletal muscle in vivo. Comp Med. **53**(2): 186-190.
14 Di A, Xu R, Peng S, Shan H, Qian Z (1997). Melatonin inhibits TRH-stimulation prolactin gene expression of anterior pituitary cells in newborn rat in vitro. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. **19**(6): 430–435.
15 Di Bella G (2008). Complete objective response to biological therapy of plurifocal breast carcinoma. Neuro Endocrinol Lett. **29**(6): 857–866.
16 Di Bella G (2011). The Di Bella Method (DBM) improved survival, objective response and performance status in a retrospective observational clinical study on 122 cases of breast cancer. Neuro Endocrinol Lett. **32**(6): 751–762.
17 Di Bella G, Colori B (2009). Complete objective response of neuroblastoma to biological treatment. Neuro Endocrinol Lett. **30**(4): 437–449.
18 Di Bella G, Colori B (2012). The Di Bella Method (DBM) improved survival, objective response and performance status in a retrospective observational clinical study on 23 tumours of the head and neck. Neuro Endocrinol Lett. **33**(3): 249–256.
19 Di Bella G, Colori B, Mascia F (2012). The Di Bella Method (DBM) improved survival, objective response and performance status in a retrospective observational clinical study on 55 cases of lymphomas. Neuro Endocrinol Lett. **33**(8): 773–781.
20 Di Bella G, Leci J, Ricchi A, Toscano R (2015). Recurrent Glioblastoma Multiforme (grade IV – WHO 2007): a case of complete objective response – concomitant administration of Somatostatin / Octreotide, Retinoids, Vit E, Vit D3, Vit C, Melatonin, D2 R agonists (Di Bella Method. Neuro Endocrinol Lett. **36**(2): 127–132.
21 Di Bella G, Madarena M (2009). Complete objective response of oesophageal squamocellular carcinoma to biological treatment. Neuro Endocrinol Lett. **30**(3): 312–321.
22 Di Bella G, Mascia F, Colori B (2013). The Di Bella Method (DBM) in the treatment of prostate cancer: a preliminary retrospective study of 16 patients and a review of the literature. Neuro Endocrinol Lett. **34**(6): 523–528.
23 Di Bella G, Mascia F, Gualano L, Di Bella L (2013). Melatonin anticancer effects: review. Int J Mol Sci. **14**(2): 2410–2430.
24 Di Bella G, Mascia F, Ricchi A, Colori B (2013). Evaluation of the safety and efficacy of the first-line treatment with somatostatin combined with melatonin, retinoids, vitamin D3, and low doses of cyclophosphamide in 20 cases of breast cancer: a preliminary report. Neuro Endocrinol Lett. **34**(7): 660–668.
25 Di Bella G, Toscano R, Ricchi A, Colori B (2016). Congenital fibrosarcoma in complete remission with Somatostatin, Bromocriptine, Retinoids, Vitamin D3, Vitamin E, Vitamin C, Melatonin, Calcium, Chondroitin sulfate associated with low doses of Cyclophosphamide in a 14-year Follow Up. Case Report. Neuro Endocrinol Lett. **36**(8): 725–733.
26 Di Bella L (1997). Melatonin from research to interventions – Acts of the conference – Reggio Calabria, Italy, Jan 01.
27 Di Bella L (1998). Cancro siamo sulla strada giusta? 1st ed. Roma. Travel Factory srl.

28 Di Bella L, Di Bella G (2015). Solution of retinoids in vitamin E in the Di Bella Method biological multitherapy. Neuro Endocrinol Lett. **36**(7): 661–676.
29 Di Bella L, Fraschini F (1997). Patent number: RE35631 – Filing date: Jun 2, 1994 – Issue date: Oct 14.
30 Di Bella L, Gualano L (2006). Key aspects of melatonin physiology: thirty years of research. Neuro Endocrinol Lett. **27**(4): 425–432.
31 Di Bella L, Rossi MT (1979). Scalera G. Perspectives in pineal function. Prog Brain Res. **52:** 475–478.
32 Di Bella L, Scalera G, Rossi MT (1980). Melatonin: An Essential Factor for the Treatment and Recovery from Leukemia and Cancer. Int Symp on Melatonin, Bremen, Germany, Sept 28–30.
33 Eck KM, Yuan L, Duffy L, Ram PT, Ayettey S, Chen I, *et al.* (1998). A sequential treatment regimen with melatonin and all-trans retinoic acid induces apoptosis in MCF-7 tumor cells. Br J Cancer. **77**(12): 2129–2137.
34 Ellis RJ1, Hartl FU (1996). Protein folding in the cell: competing models of chaperonin function. FASEB J. **10**(1): 20–26.
35 García-Mauriño S, Pozo D, Carrillo-Vico A, Calvo JR, Guerrero JM (1999). Melatonin activates Th1 lymphocytes by increasing IL-12 production. Life Sci. **65**(20): 2143–2150.
36 Griffith D, Bjoro T, Gautvik K, Haug E (1987). Melatonin reduces the production and secretion of prolactin and growth hormone from rat pituitary cells in culture. Acta Physiol Scand. **13**(1): 43–49.
37 Gyarmati G, Turner MC, Castaño-Vinyals G, Espinosa A, Papantoniou K, Alguacil J, *et al.* (2016). Night shift work and stomach cancer risk in the MCC-Spain study. Occup Environ Med. **73**(8): 520–527.
38 Hrushesky WJ, Grutsch J, Wood P, Yang X, Oh EY, Ansell C, *et al.* (2009). Circadian clock manipulation for cancer and control and the relief of cancer symptoms. Integr Cancer Ther. **8**(4): 387–397.
39 Jiang Z, Ma F (2017). Inhibitory effects of melatonin on breast cancer. Zhong Nan Da Xue Xue Bao Yi Xue Ban. **42**(3): 346–351.
40 Kauffman GB (1976). Alfred Werner's research in organic stereochemistry. Naturwissenschaften. J Chem Educ. **63**(7): 324–327.
41 Kim KJ, Choi JS, Kang I, Kim KW, Jeong CH, Jeong JW (2013). Melatonin suppresses tumor progression by reducing angiogenesis stimulated by HIF-1 in a mouse tumor model. J Pineal Res. **54**(3): 264–270.
42 Kojima T, Mochizuki C, Mitaka T, Mochizuki Y (1997). Effects of melatonin on proliferation, oxidative stress and Cx32 gap junction protein expression in primary cultures of adult rat hepatocytes. Cell Struct Funct. **22**(3): 347–356.
43 Kornblihtt LI, Finocchiaro L, Molinas FC (1993). Inhibitory effect of melatonin on platelet activation induced by collagen and arachidonic acid. J Pineal Res. **14**(4): 184–191.
44 Kvetnoy I (2002). Extrapineal melatonin in pathology: new perspectives for diagnosis, prognosis and treatment of illness. Neuro Endocrinol Lett. **23**(1): 92–96.
45 Kvetnoy I, Sandvik AK, Waldum HL (1997). The diffuse neuroendocrine system and extrapineal melatonin. J Mol Endocrinol. **18**(1): 1–3.
46 Kvetnoy IM, Kvetnaia TV, Konopljannikov AG, Tsyb AF, Yuzhakov VV (1994). Melatonin and tumour growth: from experiments to clinical application. In Kvetnoy IM, Reiter RJ (eds). Melatonin: general biological and oncoradiological aspects. MRRC Press, Obninsk, pp. 17–23.
47 Kvetnoy IM, Levin IM (1986). Melatonin and tumor growth. Eksp Onkol. **8**(4): 11–15.
48 Lemus-Wilson A, Kelly PA, Blask DE (1995). Melatonin blocks the stimulatory effects of prolactin on human breast cancer cell growth in culture. Br J Cancer. **72**(6): 1435–1440.
49 Lissoni P, Barni S, Mandalà M, Ardizzoia A, Paolorossi F, Vaghi M, *et al.* (1999). Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. Eur J Cancer. **35**(12): 1688–1692.
50 Lissoni P, Meregalli S, Nosetto L, Barni S, Tancini G, Fossati V, *et al.* (1996). Increased survival time in brain glioblastomas by a radioneuroendocrine strategy with radiotherapy plus melatonin compared to radiotherapy alone. Oncology. **53**(1): 43–46.
51 Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni GJ (2001). Antiangiogenic activity of melatonin in advanced cancer patients. Neuro Endocrinol Lett. **22**(1): 45–47.
52 Loureiro R, Magalhães-Novais S, Mesquita KA, Baldeiras I, Sousa IS, Tavares LC, *et al.* (2015). Melatonin antiproliferative effects require active mitochondrial function in embryonal carcinoma cells. Oncotarget. **6**(19): 17081–17096.
53 Maestroni GJ, Conti A, Pierpaoli W (1988). Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. Ann NY Acad Sci. **521**: 140–148.
54 Margheri M, Pacini N, Tani A, Nosi D, Squecco R, Dama A, *et al.* (2012). Combined effects of melatonin and all-trans retinoic acid and somatostatin on breast cancer cell proliferation and death: molecular basis for the anticancer effect of these molecules. Eur J Pharmacol. **681**(1–3): 34–43.
55 Martín V, Herrera F, García-Santos G, Antolin I, Rodrguez-Blanco J, Medina M (2007). Involvement of protein kinase C in melatonin's oncostatic effect in C6 glioma cells. J Pineal Res. **43**(3): 239–244.
56 Mediavilla MD, Cos S, Sánchez-Barceló EJ (1999). Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. Life Sci. **65**(4): 415–420.
57 Mediavilla MD, Güezmez A, Ramos S, Kothari L, Garijo F, Sànchez Barcelò EJ (1997). Effects of melatonin on mammary gland lesions in transgenic mice overexpressing N-ras proto-oncogene. J Pineal Res. **22**(2): 86–94.
58 Meléndez J, Maldonado V, Ortega A (1996). Effect of melatonin on beta-tubulin and MAP2 expression in NIE-115 cells. Neurochem Res. **21**(6): 653–658.
59 Meyers M, Blasi F, Bruni CB, Deeley RG, Kovach JS, Levinthal M, *et al*. (1975). Specific binding of the first enzyme for histidine biosynthesis to the DNA of histidine operon. Nucleic Acids Res. **2**(11): 2021–2036.
60 Najafi M, Shirazi A, Motevaseli E, Geraily G, Norouzi F, Heidari M *et al.* (2017). The melatonin immunomodulatory actions in radiotherapy. Biophys Rev. **9**(2): 139–148.
61 Norsa A, Martino V (2006). Somatostatin, retinoids, melatonin, vitamin D, bromocriptine, and cyclophosphamide in advanced non-small-cell lung cancer patients with low performance status. Cancer Biother Radiopharm. **21**(1): 68–73.
62 Olivieri G, Otten U, Meier F, Baysang G, Dimitriades-Schmutz B, Mùller-Spahn F, *et al.* (2003). Beta-amyloid modulates tyrosine kinase B receptor expression in SHSY5Y neuroblastoma cells: influence of the antioxidant melatonin. Neuroscience. **120**(3): 659–665.
63 Özerkan D, Özsoy N, Yılmaz E (2015). Vitamin D and melatonin protect the cell's viability and ameliorate the CCl4 induced cytotoxicity in HepG2 and Hep3B hepatoma cell lines. Cytotechnology. **67**(6): 995–1002.
64 Pauling L (1960). The Nature of the Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry. Third edition. Ithaca, NY: Cornell University Press.
65 Pawlikowski M, Winczyk K, Karasek M (2002). Oncostatic action of melatonin: facts and question marks. Neuro Endocrinol Lett. **23**(1): 24–29.
66 Reiter RJ, Tan DX, Qi W, Manchester LC, Karbownik M, Calvo JR (2000). Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol Signals Recept. **9**(3–4): 160–171.
67 Rimler A, Culig Z, Lupowitz Z, Zisapel N (2002). Nuclear exclusion of the androgen receptor by melatonin. J Steroid Biochem Mol Biol. **81**(1): 77–84.
68 Sauer LA, Dauchy RT, Blask DE (2001). Polyunsaturated fatty acids, melatonin, and cancer prevention. Biochem Pharmacol. **61**(12): 1455–1462.
69 Taysi S, Koc M, Büyükokuroğlu ME, Altinkaynak K, Sahin YN (2003). Melatonin reduces lipid peroxidation and nitric oxide during irradiation-induced oxidative injury in the rat liver. J Pineal Res. **34**(3): 173–177.
70 Tenorio Fd, Simões Mde J, Teixeira VW, Teixeira ÁA (2015). Effects of melatonin and prolactin in reproduction: review of literature. Rev Assoc Med Bras (1992). **61**(3): 269–274.

71 Teplitzky SR, Kiefer TL, Cheng Q, Dwivedi PD, Moroz K, Myers L, *et al.* (2001). Chemoprevention of NMU-induced rat mammary carcinoma with the combination of melatonin and 9-cis-retinoic acid. Cancer Lett. **168**(2): 155–163.
72 Todisco M (2009). Chronic lymphocytic leukemia: long-lasting remission with combination of cyclophosphamide, somatostatin, bromocriptine, retinoids, melatonin, and ACTH. Cancer Biother Radiopharm. **24**(3): 353–355.
73 Todisco M, Casaccia P, Rossi N (2001). Cyclophosphamide plus somatostatin, bromocriptin, retinoids, melatonin and ACTH in the treatment of low-grade non Hodgkin's lymphomas at advanced stage: results of a phase II trial. Cancer Biother Radiopharm. **16**(2): 171–177.
74 Vanecek J (1998). Cellular Mechanisms of Melatonin Action. Physiol Rev. **78**(3): 687–721.
75 Xi SC, Tam PC, Brown GM, Pang SF, Shiu SY (2000). Potential involvement of mt1 receptor and attenuated sex steroid-induced calcium influx in the direct antiproliferative action of melatonin on androgen-responsive LNCaP human prostate cancer cells. J Pineal Res. **29**(3): 172–183.
76 Zabezhinskii MA, Kovalenko AL, Kvetnoi IM, Iuzhakov VV, Popuchiev VV, Bakhtin IuB, *et al.* (1999). Effect of cycloferon on dissemination of Lewis lung carcinoma in mice and rhabdomyosarcoma ra-23 in rats. Vopr Onkol. **45**(6): 650–654.