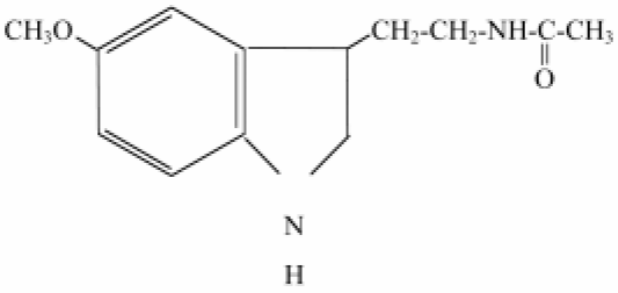
MELATONINA

IN

MALATTIE LINFOPROLIFERATIVE

N-[2-(5-Metossi-1H-indol-3-il)etil]acetamide.

$C_{13}H_{16}N_2O_2$



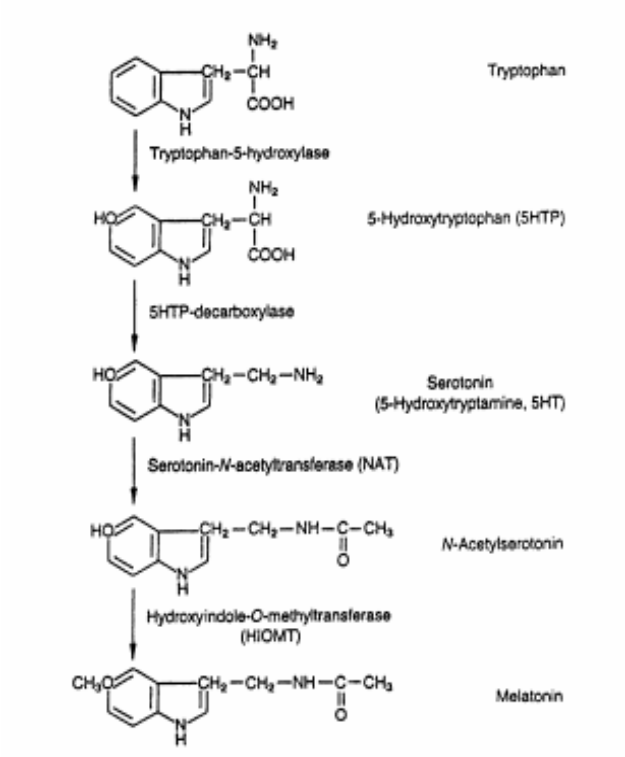
Sinonimi: N-acetil-5-metossitriptamina

Introduzione

La melatonina è stata isolata ed identificata nel 1958 da estratti di pineale bovina. La sostanza isolata fu chiamata "melatonina" per la capacità di influenzare i melanofori cutanei di anfibi e per la relazione chimica con la serotonina (5HT, 5-idrossitriptamina) (Macchi and Bruce 2004; Sugden, Davidson et al. 2004). La melatonina è distribuita ampiamente ed è stata riscontrata in organismi filogeneticamente distanti, dai batteri (Manchester, Poeggeler et al. 1995) agli umani.

La biosintesi della melatonina a partire dal triptofano coinvolge 4 passaggi intracellulari ben definiti, catalizzati dai seguenti 4 enzimi (Sugden 1989; Reiter 1991):

- triptofano idrossilasi
- decarbossilasi di amino acidi aromatici
- serotonin-N-acetiltransferasi
- idrossindol-O-metiltransferasi.



La melatonina è sintetizzata nella ghiandola pineale e la sua produzione è più alta durante la notte (Macchi and Bruce 2004). Sebbene la ghiandola pineale sia la principale fonte di melatonina, essa viene sintetizzata anche in altri siti come la retina, l'intestino ed il midollo osseo (Cagnacci 1996; Conti, Conconi et al. 2000).

Negli esseri umani, sono stati identificati due sottotipi di recettori per la melatonina, detti Mel_{1a} e Mel_{1b} oppure MT1 e MT2. Questi due recettori si trovano sulla membrana cellulare e sono accoppiati a proteine-G (Reppert, Godson et al. 1995; Slaugenhaupt, Roca et al. 1995).
Oltre che nel sistema nervoso centrale, i recettori per la melatonina umani sono stati localizzati nelle cellule epiteliali della prostata (Macchi and Bruce 2004), nello strato mucosa/submucosa del colon (Poon, Mak et al. 1996), negli spermatozoi (van Vuuren, Pitout et al. 1992), nelle cellule della granulosa provenienti da follicoli pre-ovulatori (Yie, Niles et al. 1995), nei linfociti (Lopez-Gonzalez, Calvo et al. 1992; Konakchieva, Kyurkchiev et al. 1995) e nelle piastrine ematiche (Vacas, Del Zar et al. 1992).
Anche in assenza di recettori di membrana, la molecola altamente diffusibile della melatonina esercita effetti sistemici mediante almeno due processi intracellulari:

- la modulazione delle funzioni mitotiche e citoscheletriche attraverso il legame con la calmodulina (Benitez-King and Anton-Tay 1993; Soto-Vega, Meza et al. 2004)
- agendo come uno spazzino di radicali liberi (Reiter, Tan et al. 2002).

Le funzioni della melatonina interessano molti processi fisiologici, tra cui la regolazione dei ritmi circadiani, i cambi stagionali, il sonno, la funzione riproduttiva e quella cardiovascolare (Macchi and Bruce 2004).
Inoltre, la melatonina modula anche le funzioni delle cellule del sistema immunitario ed emopoietico (Skwarlo-Sonta 2002). La melatonina stimola la piastrinogenesi ed influenza la fisiologia dei megacariociti (Lissoni, Tancini et al. 1996; Di Bella, Bruschi et al. 2002). La melatonina regola anche la produzione di citochine da parte di cellule mononucleari ematiche (Garcia-Maurino, Gonzalez-Haba et al. 1998).

Vari studi hanno mostrato che la melatonina ha proprietà oncostatiche, che possono ridurre la promozione o la progressione tumorale (Panzer and Viljoen 1997; Blask, Sauer et al. 2002; Pawlikowski, Winczyk et al. 2002). L'effetto antitumorale della melatonina può esercitarsi in vari modi.

- La melatonina attraverso i suoi effetti antiossidanti ed anti-radicali liberi può contrastare il processo di carcinogenesi (Karbownik 2002; Vijayalaxmi, Reiter et al. 2004).
- La melatonina ha effetti di potenziamento sulla risposta immune. Per esempio la co-somministrazione di melatonina ed interferon-gamma (IFN-γ) migliora la regressione tumorale nel carcinoma delle cellule renali matastatico (Neri, Fiorelli et al. 1994). Anche la co-somministarzione di melatonina e Interleuchina-2 (IL-2) è efficace in pazienti con neoplasie solide avanzate (Lissoni, Barni et al. 1993).

- La melatonina ha effetti protettivi sulla tossicità indotta da farmaci chemioterapici, potenziandone così la loro efficacia clinica (Anwar, Mahfouz et al. 1998; Rapozzi, Zorzet et al. 1998). È stato anche mostrato che la chemioterapia causa un'evidente diminuzione dei livelli sierici di melatonina (Lissoni, Bastone et al. 1987).
- L'effetto oncostatico della melatonina è abbastanza pronunciato in tumori dipendenti da ormoni riproduttivi (come seno ed ovaio), indicando che la melatonina può antagonizzare la mitogenesi indotta da estrogeni (Hill, Spriggs et al. 1992).
- Infine, è stato mostrato che la melatonina può controllare la crescita tumorale anche agendo come una molecola naturale anti-angiogenica, contrastando così l'angiogenesi dipendente dalla proliferazione delle cellule maligne (Lissoni, Rovelli et al. 2001).

Melatonina in malattie linfoproliferative

È stato mostrato che la melatonina gioca un ruolo fondamentale nel processo di neuro-immuno-modulazione (Guerrero and Reiter 1992; Skwarlo-Sonta 2002).
L'esistenza di specifici recettori per la melatonina in cellule linfoidi indica un diretto effetto della melatonina nella regolazione del sistema immune (Gonzalez-Haba, Garcia-Maurino et al. 1995; Garcia-Perganeda, Pozo et al. 1997).
Studi in vivo dimostrano che la melatonina esercita proprietà immunostimolanti. Infatti, la pinealectomia in ratti neonati causa disorganizzazione della struttura timica e la luce costante diminuisce la risposta anticorpale delle cellule T dipendente da antigeni (Jankovic, Isakovic et al. 1970; Csaba and Barath 1975). Inoltre, la pinealectomia inibisce la produzione di IL-2 e l'attività delle cellule Natural Killer (NK) (del Gobbo, Libri et al. 1989). Invece, il trattamento con melatonina potenzia la citotossicità cellulare anticorpo-dipendente (Giordano and Palermo 1991) e la produzione di IFN-γ da splenociti murini (Colombo, Chen et al. 1992). La proliferazione di linfociti, in vitro, è anche inibita dalla melatonina (Markowska, Waloch et al. 2001).

Studi in vitro mostrano che la melatonina agisce sulle cellule immuni attraverso la regolazione della produzione di citochine. Infatti, la melatonina attiva le cellule T-helper mediante l'aumento della produzione di IL-2, così come attivando i monociti attraverso l'aumento della produzione di IL-1, IL-6, TNF (tumor necrosis factor), ROS (reactive oxygen species) e NO (nitric oxide) (Morrey, McLachlan et al. 1994; Garcia-Maurino, Gonzalez-Haba et al. 1997; Barjavel, Mamdouh et al. 1998). La melatonina potenzia anche la produzione di IL-12 da parte dei monociti inducendo il differenziamento delle cellule-T verso il fenotipo Th1 e causando un aumento della produzione di IFN-γ (Garcia-Maurino, Pozo et al. 1999).
Quindi, la produzione di citochine può essere considerata come uno dei principali meccanismi per modulare il sistema immune da parte della melatonina.

Questi effetti regolativi sul sistema immune non derivano solo dalla melatonina sintetizzata dalla ghiandola pineale. Infatti, è stato dimostrato che anche i linfociti umani sintetizzano melatonina, indicando così i linfociti come un'importante fonte fisiologica di melatonina (Carrillo-Vico, Calvo et al. 2004). Inoltre, lo stesso studio ha riportato che la melatonina sintetizzata dai linfociti è coinvolta nella modulazione dell'IL-2, aumentandone la produzione. Quindi, questi risultati indicano che la melatonina può regolare il sistema immune umano agendo come una sostanza intracrina, autocrina e/o paracrina (Carrillo-Vico, Calvo et al. 2004).

Questi dati indicano un possibile effetto benefico della melatonina in pazienti con malattie linfoproliferative. Uno studio clinico ha valutato la concomitante somministrazione di melatonina con basse dosi di IL-2 in pazienti con neoplasie ematologiche avanzate non trattabili con altre terapie. I risultati di tale studio indicano che la combinazione melatonina più IL-2 può prolungare il tempo di sopravvivenza in questi pazienti, con risultati comparabili a quelli precedentemente riportati usando una immunoterapia più tossica, consistente di alte dosi di IL-2 da sola (Lissoni, Bolis et al. 2000).

Uno studio clinico ha valutato la melatonina in combinazione con somatostatina, bromocriptina, retinoidi, ciclofosfamide e ACTH nel trattamento di linfomi non-Hodgkin a basso grado in fase avanzata (Todisco, Casaccia et al. 2001). Su 20 pazienti valutabili per risposta e tossicità il 70% (14 su 20) ebbe una risposta parziale; il 20% (4 su 20) ebbe una malattia stabile ed il 10% (2 su 20) ebbe progressione di malattia. Continuando con la terapia, nessuno dei 14 pazienti con una risposta parziale ebbe una progressione della malattia (tempo di controllo medio di 21 mesi, intervallo da 7 a 25) ed il 50% di questi pazienti ebbe una risposta completa. Dei 4 pazienti con malattia stabile, il 25% (1 di 4) ebbe una risposta parziale ed il 75% (3 di 4) progredirono con la terapia (tempo medio di progressione 14.3 mesi, intervallo da 7 a 21). La tossicità era molto modesta, gli effetti collaterali più comuni furono: sonnolenza, diarrea e iperglicemia.
Questo studio conferma che in neoplasie ematologiche, l'utilizzo della melatonina, associata ad altre molecole efficaci in tali neoplasie, può essere di utilità terapeutica e supporta il razionale scientifico del Metodo Di Bella.

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Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs.

Anwar MM, Mahfouz HA, Sayed AS.

Myelosuppression is the most serious, dose limiting, toxicity of cytotoxic drugs. Efforts to protect the bone marrow have been only variably successful, and no agreement exists on how to approach this problem. Melatonin, the major hormonal product of the pineal gland, is supposed to have both chemoprotective and myelostimulatory effects. This experimental study was carried out to test these two effects on the bone marrow of rats, daily intraperitoneally injected with 100 microg melatonin. Injection of 10 mg aracytin for 10 days produced a significant ($P < 0.01$) decrease in red blood cells count (RBCs), total leucocytic count, as well as platelets count. When melatonin was injected along with aracytin, it would significantly increase ($P < 0.05$) RBC count and ($P < 0.01$) blood platelet count. Injection of melatonin after aracytin treatment would significantly increase ($P < 0.01$) RBC, total leucocytic and platelet counts in comparison with rats treated with aracytin only. The effects of melatonin were more clear in rats treated with it after aracytin injection than those treated with melatonin and aracytin at the same time. Furthermore, it was found that aracytin produced a significant ($P < 0.01$) decrease in serum total proteins, albumin, and significantly increased the ($P < 0.01$) albumin/globulin ratio. Melatonin injection would significantly increase ($P < 0.01$) total protein, globulin, and significantly decrease ($P < 0.01$) the albumin/glubulin ratio when injected either with aracytin or after aracytin treatment. These results indicate that melatonin protects bone marrow, lymphoid tissues from damaging effect of cytotoxic drugs, as well as stimulating the suppressed bone marrow.

J Immunol. 1998 Feb 1;160(3):1191-7.

Differential expression of the melatonin receptor in human monocytes.

Barjavel MJ, Mamdouh Z, Raghbate N, Bakouche O.

Earlier studies have shown that the pineal hormone melatonin activates human monocytes. It is reported here that melatonin induces the secretion of IL-1, IL-6, and TNF in fresh and 1-day in vitro cultured monocytes that also express the melatonin receptor (Kd = 270 +/- 60 pM; 42,000-48,000 receptors/cell). However, when monocytes were cultured in vitro for 2 days, the number of receptors decreased to 11,000 receptors/cell, with the same Kd. LPS activation of fresh or 1-day cultured monocytes did not result in any increase in melatonin receptor number. LPS activation of 2-day cultured monocytes led to an increase in the number of melatonin receptors, from 11,000 receptors/cell to the plateau of 42,000 to 48,000 receptors/cell. The loss of receptors by 2-day cultured monocytes was not irreversible. Melatonin did not induce the release of IL-1, TNF, or IL-6 in monocytes cultured in vitro for 3 days and for up to 15 days, and these long time cultured monocytes did not express the melatonin receptors even after activation by LPS. The loss of melatonin receptors by monocytes cultured in vitro for 3 days and for up to 15 days was irreversible. Therefore, it is shown for the first time that human monocytes express melatonin receptors. Furthermore, human monocytes express melatonin receptors differentially depending on their state of maturation, and it appears that in vitro monocyte differentiation and maturation negatively affect human monocyte melatonin receptor expression.

Experientia. 1993 Aug 15;49(8):635-41.

Calmodulin mediates melatonin cytoskeletal effects.

Benitez-King G, Anton-Tay F.

In this article, we review the data concerning melatonin interactions with calmodulin. The kinetics of melatonin-calmodulin binding suggest that the hormone modulates cell activity through intracellular binding to the protein at physiological concentration ranges. Melatonin interaction with calmodulin may allow the hormone to modulate rhythmically many cellular functions. Melatonin's effect on tubulin polymerization, and cytoskeletal changes in MDCK and N1E-115 cells cultured with melatonin, suggest that at low concentrations (10(-9) M) cytoskeletal effects are mediated by its antagonism to Ca2+-calmodulin. At higher concentrations (10(-5)M) non-specific binding of melatonin to tubulin occurs thus overcoming the specific melatonin antagonism to Ca2+-calmodulin. Since the structures of melatonin and calmodulin are phylogenetically well preserved, calmodulin-melatonin interaction probably represents a major mechanism for regulation and synchronization of cell physiology.

Curr Top Med Chem. 2002 Feb;2(2):113-32.

Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy.

Blask DE, Sauer LA, Dauchy RT.

Melatonin, as a new member of an expanding group of regulatory factors that control cell proliferation and loss, is the only known chronobiotic, hormonal regulator of neoplastic cell growth. At physiological circulating concentrations, this indoleamine is cytostatic and inhibits cancer cell proliferation in vitro via specific cell cycle effects. At pharmacological concentrations, melatonin exhibits cytotoxic activity in cancer cells. At both physiological and pharmacological concentrations, melatonin acts as a differentiating agent in some cancer cells and lowers their invasive and metastatic status through alterations in adhesion molecules and maintenance of gap junctional intercellular communication. In other cancer cell types, melatonin, either alone or in combination with other agents, induces apoptotic cell death. Biochemical and molecular mechanisms of melatonin's oncostatic action may include regulation of estrogen receptor expression and transactivation, calcium/calmodulin activity, protein kinase C activity, cytoskeletal architecture and function, intracellular redox status, melatonin receptor-mediated signal transduction cascades, and fatty acid transport and metabolism. A major mechanism mediating melatonin's circadian stage-dependent tumor growth inhibitory action is the suppression of epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK) activity. This occurs via melatonin receptor-mediated blockade of tumor linoleic acid uptake and its conversion to 13-hydroxyoctadecadienoic acid (13-HODE) which normally activates EGFR/MAPK mitogenic signaling. This represents a potentially unifying model for the chronobiological inhibitory regulation of cancer growth by melatonin in the maintenance of the host/cancer balance. It also provides the first biological explanation of melatonin-induced enhancement of the efficacy and reduced toxicity of chemo- and radiotherapy in cancer patients.

J Pineal Res. 1996 Nov;21(4):200-13.

Melatonin in relation to physiology in adult humans.

Cagnacci A.

The role exerted by melatonin in human physiology has not been completely ascertained. Melatonin levels have been measured in different physiopathological conditions, but the effects induced by melatonin administration or withdrawal have been tested only recently. Some effects have been clearly documented. Melatonin has hypothermic properties, and its nocturnal secretion generates about 40% of the amplitude of the circadian body temperature rhythm. Melatonin has sleep inducing properties, and exerts important activities in the regulation of circadian rhythms. Melatonin is capable of phase shifting human circadian rhythms, of entraining free-running circadian rhythms, and of antagonizing phase shifts induced by nighttime exposure to light. Its effect on human reproduction is not completely clear, but stimulatory effects on gonadotropin secretion have been reported in the follicular phase of the menstrual cycle. Direct actions on ovarian cells and spermatozoa have been also documented. Beside these, new important actions for melatonin may be proved. Melatonin may exert protective effects on the cardiovascular system, by reducing the risk of atherosclerosis and hypertension, and may influence immune responses. Finally, by acting as an antioxidant, melatonin could be important in slowing the processes of ageing.

FASEB J. 2004 Mar;18(3):537-9. Epub 2004 Jan 8.

Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance.

Carrillo-Vico A, Calvo JR, Abreu P, Lardone PJ, Garcia-Maurino S, Reiter RJ, Guerrero JM.

It has been historically assumed that the pineal gland is the major source of melatonin (N-acetyl-5-methoxytryptamine) in vertebrates. Melatonin plays a central role in fine-tuning circadian rhythms in vertebrate physiology. In addition, melatonin shows a remarkable functional versatility exhibiting antioxidant, oncostatic, antiaging, and immunomodulatory properties. Melatonin has been identified in a wide range of organisms from bacteria to human beings. Its biosynthesis from tryptophan involves four well-defined intracellular steps catalyzed by tryptophan hydroxylase, aromatic amino acid decarboxylase, serotonin-N-acetyltransferase, and hydroxyindole-O-methyltransferase. Here, for the first time, we document that both resting and phytohemagglutinin-stimulated human lymphocytes synthesize and release large amounts of melatonin, with the melatonin concentration in the medium increasing up to five times the nocturnal physiological levels in human serum. Moreover, we show that the necessary machinery to synthesize melatonin is present in human lymphocytes. Furthermore, melatonin released to the culture medium is synthesized in the cells, because blocking the enzymes required for its biosynthesis or inhibiting protein synthesis in general produced a significant reduction in melatonin release. Moreover, this inhibition caused a decrease in IL-2 production, which was restored by adding exogenous melatonin. These findings indicate that in addition to pineal gland, human lymphoid cells are an important physiological source of melatonin and that this melatonin could be involved in the regulation of the human immune system, possibly by acting as an intracrine, autocrine, and/or paracrine substance.

Immunol Lett. 1992 Jul;33(2):123-6.

Melatonin induced increase in gamma-interferon production by murine splenocytes.

Colombo LL, Chen GJ, Lopez MC, Watson RR.

Previously, we demonstrated that production of gamma-interferon (gamma-IFN) by the mouse splenocytes isolated at night was higher than from those isolated in the morning. In this paper we show that melatonin increased gamma-IFN production by murine splenocytes. Moreover, this stimulating effect was significantly higher (10 times) in the cells isolated at night than in those isolated in the morning (2 times).

J Pineal Res. 2000 May;28(4):193-202.

Evidence for melatonin synthesis in mouse and human bone marrow cells.

Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM.

Recently, it was demonstrated that inbred strains of mice have a clearcut circadian rhythm of pineal and serum melatonin. Moreover, it is known that melatonin is involved in many immunoregulatory functions. Among them, hematopoiesis is influenced by the action of melatonin via melatonin-induced opioids on kappa-opioid receptors, which are present on stromal bone marrow cells. Therefore, the present study was carried out to investigate the presence of melatonin in the bone marrow in which immunocompetent cells are generated. Specifically, we aimed at answering the following question: are bone marrow cells involved in melatonin synthesis? In the present study, we demonstrate that (1) bone marrow cells contain high concentrations of melatonin; (2) bone marrow cells have a N-acetyltransferase activity and they express the mRNA encoding hydroxy-O-methyltransferase and (3) bone marrow cells cultured for a prolonged period exhibited high levels of melatonin. Results presented here suggest that mouse and human bone marrow and bone marrow cells are capable of de novo synthesis of melatonin, which may have intracellular and or paracrine functions.

Endocrinol Exp. 1975 Jan;9(1):59-67.

Morphological changes of thymus and the thyroid gland after postnatal extirpation of pineal body.

Csaba G, Barath P.

Extirpation of the pineal body of newborn rats was followed by the disorganization of thymic structure, follicular transformation and proliferation, viz. malignant transformation of its epithelial and connective tissue elements. Increase of connective tissue was also observed in the thyroid gland. The follicular cells contained crystal-like bodies and vesicles, whereas in the parafollicular cells the endoplasmic reticulum was increased and light granules appeared.

Int J Immunopharmacol. 1989;11(5):567-73.

Pinealectomy inhibits interleukin-2 production and natural killer activity in mice.

del Gobbo V, Libri V, Villani N, Calio R, Nistico G.

Four--five-week-old C57BL/6 mice were surgically pinealectomized. At different time intervals after surgery their spleens were removed and assayed for interleukin-2 (IL-2) production and natural killer (NK) cell activity. Non-operated and sham-operated mice were used as controls. The present results indicate that pinealectomy significantly reduced IL-2 production and NK cell activity, in comparison to sham-operated mice. These effects seem to be related to the lack of melatonin. In fact the subcutaneous injection of this hormone (50 or 100 mg/kg at 5 p.m.) in pinealectomized mice was able to restore IL-2 production and NK cell activity. However, chronic treatment with melatonin (10, 20 and 50 mg/kg for 9 consecutive days) failed to reverse the impairment of the immune responses.

Med Sci Monit. 2002 Dec;8(12):BR527-31.

Melatonin effects on megakaryocyte membrane patch-clamp outward K+ current.

Di Bella L, Bruschi C, Gualano L.

BACKGROUND: This study was carried out to evaluate the influence of melatonin concentration on rat bone marrow megakaryocyte outward K+ current and its implications with regard to platelet production. It is the Authors' view that the greatly extended development of megakaryocyte membrane, together with its ion channels, makes the choice of this topic particularly pertinent. MATERIAL/METHODS: Megakaryocytes from fresh Wistar rat bone marrow were clampaged with patch-clamp technique and the examination of membrane outward current was performed when melatonin dissolved in external or internal standard solution was used. RESULTS: On the basis of this study, melatonin does reduce outward K+ current intensity, the more the higher the melatonin concentration. In quantitative terms, whereas no change is noticed when dissolved melatonin concentration in external standard solution is smaller than 25 mM, at 50 mM only a delayed outward current decrease appears. The effect on the outward current intensity is reversible at least until melatonin concentration reaches 500 mM; when melatonin concentration is higher than 1000 mM the effect is demonstrably irreversible. The presence on the megakaryocyte membrane of internal standard melatonin solution does reduce the outward current even more sharply. CONCLUSIONS: There seems to be a positive correlation between cationic pump and megakaryocyte intimate processes of platelet production.

J Immunol. 1997 Jul 15;159(2):574-81.

Melatonin enhances IL-2, IL-6, and IFN-gamma production by human circulating CD4+ cells: a possible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes.

Garcia-Maurino S, Gonzalez-Haba MG, Calvo JR, Rafii-El-Idrissi M, Sanchez-Margalet V, Goberna R, Guerrero JM.

This paper shows that melatonin is able to activate human Th1 lymphocytes by increasing the production of IL-2 and IFN-gamma in vitro. Th2 cells appear not to be affected by melatonin, since IL-4, which is mostly produced by Th2 cells, is not modified by the hormone. Melatonin also enhances IL-6 production by PBMCs. The activation by melatonin of IL-6 production is apparently related to the presence of monocytes, rather than to Th2 cells, in the cell preparation, since PBMCs depleted of monocytes (CD14+ cells) were not activated. Activation of PBMCs by melatonin was dependent on the dose and, measured by cytokine production, was observed only when cells were either not activated or only slightly activated by low concentrations of PHA, or when cell activation was achieved by incubating the cells with previously irradiated cells. Using a different approach to identify what type of cells among the PBMC subsets was activated by melatonin, the expression of CD69, a marker of cell activation, was studied. Melatonin increased the percentage of cells expressing the CD69 Ag in CD4+ but not in CD8+ cells. We have also achieved enhanced production of IL-2 and IL-6 using CGP 52608, a specific ligand of the putative nuclear melatonin receptor RZR/ROR, raising the possibility of direct effects of melatonin on gene regulation in both Th1 cells and monocytes. The results suggest that melatonin may be involved in the regulation of human immune functions by modulating the activity of Th1 cells and monocytes via nuclear receptor-mediated transcriptional control.

J Neuroimmunol. 1998 Dec 1;92(1-2):76-84.

Involvement of nuclear binding sites for melatonin in the regulation of IL-2 and IL-6 production by human blood mononuclear cells.

Garcia-Maurino S, Gonzalez-Haba MG, Calvo JR, Goberna R, Guerrero JM.

Many functional studies show that melatonin plays a fundamental role in neuroimmunomodulation. In this paper, we have extended our studies on the influence of melatonin on IL-2 and IL-6 production by human peripheral blood mononuclear cells (PBMCs) by comparing the effects of the specific membrane receptor agonist S 20098, the RZR/ROR(alpha) receptor agonist CGP 52608, and structurally related thiazolidinediones. Melatonin bound to membranes as well as to nuclei of human PBMCs with about the same affinity (IC50 values around 5 nM). S 20098 bound to PBMC membranes but not to PBMC nuclei, although the affinity was at least 100 times lower than that of melatonin; this compound did not stimulate cytokine production. In contrast, all four CGP compounds did not bind to PBMC membranes, while binding to nuclei exhibited IC50 values comparable to those of melatonin. The thiazolidinediones activating the RZR/ROR(alpha) receptor (CGP 52608, CGP 53079) also increased IL-2 and IL-6 production. CGP 55644 had no effect on cytokine production and antagonized the effects of CGP 52608 on IL-2 and IL-6 production; moreover, CGP 55644 decreased the enhanced IL-2 production caused by melatonin. Results obtained in monocyte cultures resembled closely those shown in PBMCs. The results reported in this paper confirm the involvement of a nuclear mechanism in the melatonin effects on cytokine production in human PBMCs. We have also shown a synergistic effect of S 20098 and CGP 52608, suggesting a possible link between nuclear and membrane melatonin receptors in PBMCs.

Life Sci. 1999;65(20):2143-50.

Melatonin activates Th1 lymphocytes by increasing IL-12 production.

Garcia-Maurino S, Pozo D, Carrillo-Vico A, Calvo JR, Guerrero JM.

Melatonin could act on immune system by regulating cytokine production of immunocompetent cells. The hormone enhances IL-2, IFN-gamma and IL-6 production by cultured human mononuclear cells. As enhancement of IL-6 production is related to monocyte activation by melatonin, the hormone acts on human lymphoid cells causing a Th1-type response. This paper shows that melatonin seems to promote a Th1-response by increasing IL-12 production. The hormone enhances IL-12 production by cultured monocytes under suboptimal stimulation in a dose-dependent way. The effect of the hormone increases when PBMCs are incubated with melatonin before monocyte isolation. Enhanced IL-12 production by melatonin can also be shown in cultured human mononuclear cells.

J Immunol. 1997 Oct 15;159(8):3774-81.

Signal transduction for melatonin in human lymphocytes: involvement of a pertussis toxin-sensitive G protein.

Garcia-Perganeda A, Pozo D, Guerrero JM, Calvo JR.

We analyzed the melatonin signal transduction in human blood lymphocytes. High affinity melatonin receptors were identified by specific binding of 2-[125I]melatonin ([125I]MEL) to human lymphocyte membranes. Scatchard analysis of [125I]MEL binding revealed high affinity receptors, with a dissociation constant (Kd) of 0.45 nM and a binding capacity (Bmax) of 7.8 fmol/mg protein. Specific [125I]MEL binding was reduced markedly by GTP and its nonhydrolyzable analogues guanosine 5'-beta, gamma-imidotriphosphate (Gpp(NH)p) and guanosine 5'-O-(3-triphosphate) (GTP-gamma-S). Gpp(NH)p inhibited in a dose-dependent manner the [125I]MEL specifically bound to human lymphocyte membranes with a half-maximal inhibition (IC50) of 3.5 +/- 0.6 microM Gpp(NH)p. Treatment of human lymphocyte membranes with Gpp(NH)p increased the Kd value (Kd = 1.20 nM). Melatonin inhibited significantly and in a dose-dependent manner forskolin-stimulated cAMP production in intact human lymphocytes. Melatonin was able to stimulate diacylglycerol production in a dose-dependent manner in human lymphocyte membranes. Pertussis toxin treatment inhibited the specific [125I]MEL binding and blocked the ability of melatonin to both inhibit forskolin-stimulated cAMP production and stimulate diacylglycerol production. Pertussis toxin ADP-ribosylation and Western blot experiments demonstrated the protein expression of alpha i1/2, alpha i3/0, and beta gamma complexes of G proteins in human lymphocyte membranes. The results strongly suggest a pertussis toxin-sensitive melatonin signal transduction pathway in human lymphocytes that involves the inhibition of adenylyl cyclase and the stimulation of phospholipase C.

J Pineal Res. 1991 Apr;10(3):117-21.

Melatonin-induced enhancement of antibody-dependent cellular cytotoxicity.

Giordano M, Palermo MS.

Antibody-dependent cellular cytotoxicity (ADCC) is a lytic mechanism in which a specific antibody acts cooperatively with leukocytic effector cells to induce target cell lysis. In this paper, the effect of exogenous melatonin on ADCC was examined. It was found that two evening intravenous injections of melatonin (1 mg/kg b.w.) was sufficient to enhance the capacity of splenocytes to mediate ADCC. This augmented activity returned to normal levels by day 6. Moreover, the opioid antagonist, naloxone, was unable to inhibit the ADCC enhancement, suggesting that melatonin did not operate through a naloxone-sensitive opiatergic mechanism. These results further support the modulatory action of melatonin on immune responses.

FASEB J. 1995 Oct;9(13):1331-5.

High-affinity binding of melatonin by human circulating T lymphocytes (CD4+).

Gonzalez-Haba MG, Garcia-Maurino S, Calvo JR, Goberna R, Guerrero JM.

This paper shows the presence of high-affinity binding sites for melatonin in human circulating T lymphocytes, but not in B lymphocytes. The binding of melatonin to T cells was dependent on time, stable, reversible, saturable, specific, and inversely correlated to the production of melatonin, expressed as the nocturnal 12 h production of its urinary metabolite 6-sulfatoxymelatonin. The affinity of these binding sites (Kd = 0.27 nM) suggests that they may recognize the physiological concentrations of melatonin in serum. Moreover, among the lymphocyte subpopulations studied, binding of melatonin was mostly found in CD4+ cells rather than in CD8+ cells. Results suggest that CD4+ cells may be the target of melatonin among the human circulating lymphocytes.

Endocr Res. 1992;18(2):91-113.

A brief survey of pineal gland-immune system interrelationships.

Guerrero JM, Reiter RJ.

The present paper summarizes evidence that support the hypothesis of the existence of bilateral interactions between pineal gland and the immune system. Both in vivo and in vitro experiments show that the pineal gland, via its hormone melatonin, enhances immune function. Mechanisms involved in this immunostimulatory effect are not well understood, but some evidence suggests the existence of specific binding sites for melatonin on immune cells. Moreover, the release of opioid peptides and interleukin-2 by T-helper cells may also participate in this mechanism by activating, at least natural killer activity and antibody-dependent cellular cytotoxicity. Some immune signals, i.e., gamma-interferon, may be involved in regulating pineal function, thereby representing a regulatory mechanism in the opposite direction. The physiological and clinical significance of these data remains to be studied.

Cancer Lett. 1992 Jul 10;64(3):249-56.

The growth inhibitory action of melatonin on human breast cancer cells is linked to the estrogen response system.

Hill SM, Spriggs LL, Simon MA, Muraoka H, Blask DE.

The pineal hormone, melatonin, was examined for its capacity to modulate the proliferation of a panel of human breast cancer cell lines. Melatonin inhibited, to a varying extent, the proliferation of all three estrogen-responsive cell lines, but had no effect on estrogen-insensitive breast tumor cell lines. Melatonin was also able to specifically block estrogen-induced proliferation in MCF-7 breast cancer cells. However, this action was abolished in the presence of tamoxifen. Therefore, it appears that the antiproliferative effects of melatonin are mediated through the estrogen-response pathway.

Neuro Endocrinol Lett. 2002 Apr;23 Suppl 1:39-44.

Potential anticarcinogenic action of melatonin and other antioxidants mediated by antioxidative mechanisms.

Karbownik M.

The complex process of carcinogenesis is, to a large extent, due to oxidative stress. Numerous indicators of oxidative damage are enhanced in the result of the action of carcinogens. Several antioxidants protect, with different efficacy, against oxidative abuse, exerted by carcinogens. Recently, melatonin (N-acetyl-5-methoxytryptamine) and some other indoleamines have gained particular meaning in the defense against oxidative stress and, consequently, carcinogenesis. Some antioxidants, like ascorbic acid, play a bivalent role in the antioxidative defense, revealing, under specific conditions, prooxidative effects. Among known antioxidants, melatonin is particularly frequently applied in experimental models of anticarcinogenic action. In the numerous studies, examining several parameters of oxidative damage and using several in vitro and in vivo models, this indoleamine has been shown to protect DNA and cellular membranes from the oxidative abuse caused by carcinogens. When either preventing or decreasing the oxidative damage to macromolecules, melatonin also protects against the initiation of cancer. The protection provided by melatonin and some other antioxidants against cellular damage, due to carcinogens, make them potential therapeutic supplements in the conditions of increased cancer risk.

J Neuroimmunol. 1995 Dec 31;63(2):125-32.

Selective effect of methoxyindoles on the lymphocyte proliferation and melatonin binding to activated human lymphoid cells.

Konakchieva R, Kyurkchiev S, Kehayov I, Taushanova P, Kanchev L.

Three pineal methoxyindoles (melatonin (Mel), 5-methoxytryptamine (5-MTA) and 5-methoxytryptophol (5-MTO)) were studied for their ability to influence the proliferative response of human peripheral blood lymphocytes (PBL) and tonsillar lymphocytes (TL) following activation with concanavalin A (ConA) in vitro. The ConA-stimulated DNA synthesis was affected in a different dose-dependent mode by the methoxyindoles tested. Melatonin and 5-MTO inhibited and 5-MTA increased the ConA-induced [3H]thymidine incorporation in PBL and TL. The initial screening for 2-[125I]iodomelatonin binding using a single point assay revealed significantly increased specific binding to PBL and TL after 72-h stimulation with ConA as compared to the non-activated cell cultures. Coincubation of separate lymphocyte cultures with ConA and Mel or 5-MTO resulted in inhibition of the specific 2-[125I]iodomelatonin binding (85% and 74%, respectively). The specific binding determined in the presence of 5-MTA did not differ from control values. Series of saturation and competition experiments were performed to examine the binding characteristics of ConA-stimulated lymphocytes for 2-[125I]iodomelatonin. The radioligand labelled binding sites of high affinity (Kd = 0.14 +/- 0.03 nM) and low capacity (Bmax = 6.8 +/- 1.5 fM/mg protein). Competitive studies with a variety of indoles determined the following order of relative potency for inhibition of 2-[125I]iodomelatonin binding in TL: 2-iodomelatonin > melatonin > > 5-methoxytryptophol. 5-Methoxytryptamine did not show displacement potency for the labelled ligand. Collectively, our data suggest that pineal hormones might be directly involved in the regulation of the T-lymphoproliferative response of human lymphoid cells. We show the availability of melatonin receptors, which seem to be an intrinsic characteristic of activated human lymphocyte populations. While the effects of Mel and 5-MTO can be linked to the binding sites described, it is unlikely that serotonin agonists like 5-MTA may act through the same sites to influence the mitogen-stimulated lymphocyte proliferation.

Eur J Cancer Clin Oncol. 1987 Jul;23(7):949-57.

The clinical significance of melatonin serum determination in oncological patients and its correlations with GH and PRL blood levels.

Lissoni P, Bastone A, Sala R, Mauri R, Rovelli F, Viviani S, Bajetta E, Esposti D, Esposti G, di Bella L, et al.

In order to investigate the pineal function and its relation with the hypophysis in human neoplasms, melatonin and GH serum levels were determined in 63 patients, 42 affected by solid tumours and 21 by lymphoma or leukaemia. In women with breast cancer PRL was also measured. Melatonin, GH and PRL were evaluated in 52 healthy subjects acting as controls. The oncological patients showed significantly higher mean melatonin serum levels than the control subjects. Mean melatonin values were lower in patients with solid tumours who had metastases, than in cases without metastases. Chemotherapy caused an evident decrease in melatonin levels. Surgery was followed by a fall in melatonin in patients without metastases. Mean GH serum levels observed in oncological patients were similar to those in control subjects and were not influenced by therapy. PRL levels were within the normal range in women suffering from breast cancer.

Eur J Cancer. 1993;29A(2):185-9.

Neuroimmunotherapy of advanced solid neoplasms with single evening subcutaneous injection of low-dose interleukin-2 and melatonin: preliminary results.

Lissoni P, Barni S, Rovelli F, Brivio F, Ardizzoia A, Tancini G, Conti A, Maestroni GJ.

On the basis of the demonstrated existence of immunoneuroendocrine interactions and on the previously observed synergistic action between the pineal hormone melatonin (MLT) and interleukin-2 (IL-2), we have designed a neuroimmunotherapeutic combination consisting of low-dose IL-2 and MLT in the treatment of advanced solid neoplasms. The study included 24 patients with advanced solid tumours (non-small cell lung cancer 9; colorectal cancer 7; gastric cancer 3; breast cancer 2; cancer of pancreas 1; hepatocarcinoma 1; unknown primary tumour 1), 21 of whom showed distant organ metastases. Not all patients responded to previous chemotherapies, or had tumours for which no standard therapy was available. Moreover, not all patients were able to tolerate IL-2 immunotherapy at the conventional doses. IL-2 was given subcutaneously at a dose of 3 x 10(6) U/day at 8:00 p.m. for 6 days/week for 4 weeks. MLT was given orally at a dose of 50 mg at 8:00 p.m. every day, starting 7 days before IL-2 injection. In non-progressed patients, a second cycle was given after a 21-day rest period. A partial response was seen in 3/24 patients (lung 2; stomach 1; duration: 11, 4, 4 months, respectively). Moreover, a minimal response (duration: 8+ months) was seen in 1 lung cancer patient. Stable disease was obtained in 14/24 patients (median duration: 6+ months), while the remaining 6 patients progressed. An improvement in performance status was seen in 7/24 patients. No important toxicity was observed. Mean eosinophil and lymphocyte levels significantly increased during the immunotherapy, and their rise was significantly higher in patients with response or stable disease than in those with progressive disease. These preliminary results show that neuroimmunotherapy with low-dose IL-2 and the pineal hormone MLT is a biologically active and well tolerated strategy, capable of determining an apparent control of tumour growth in patients with advanced solid neoplasms, for whom no standard effective therapy is available.

Recenti Prog Med. 1996 Dec;87(12):582-5.

The pineal hormone melatonin in hematology and its potential efficacy in the treatment of thrombocytopenia.

Lissoni P, Tancini G, Barni S, Paolorossi F, Rossini F, Maffe P, Di Bella L.

Recent experimental studies suggested that hematopoietic cell proliferation and differentiation are under a neuroendocrine control and that they change in relation to the 24-hour period. Moreover, it has been shown that the pineal hormone melatonin (MLT) plays a role in mediating the influence of the psychoendocrine system and of the lighting conditions on the hematopoiesis. Finally, MLT has appeared to regulate hematopoietic cell growth by influencing apoptosis-related mechanisms. In particular, preliminary studies have shown that the pineal hormone MLT may determine some benefits in blood cell disorders, mainly platelet diseases. On this basis, a pilot phase II study of MLT therapy was performed in patients suffering from persistent thrombocytopenia due to different causes. The study included 14 patients, and thrombocytopenia was due to bone metastatic involvement in 5, hypersplenism in 3, myelodysplastic syndrome in 3, DIC in 1, genetic factors in 1, and Werlhof's disease in the last case. MLT was given orally at 20 mg/day in the evening for 2 months. No MLT-related toxicity occurred. A normalization of platelet number was achieved in 8/14 (57%), and platelet mean number significantly increased on MLT therapy. This preliminary study would suggest that MLT may be effective in the treatment of thrombocytopenia due to different reasons, for which no effective standard therapy is available.

Anticancer Res. 2000 May-Jun;20(3B):2103-5.

A phase II study of neuroimmunotherapy with subcutaneous low-dose IL-2 plus the pineal hormone melatonin in untreatable advanced hematologic malignancies.

Lissoni P, Bolis S, Brivio F, Fumagalli L.

Interleukin-2 (IL-2) has proven to be able to generate an effective anticancer immunity against both solid and hematologic malignancies. Moreover, recent advances in the knowledge of psychoneuroimmunology have demonstrated that anticancer immunity is under neuroendocrine control and that the pineal hormone melatonin (MLT) may stimulate the IL-2-dependent anticancer reaction. Finally, preliminary clinical studies have already shown that the concommitant administration of MLT may amplify the efficacy of IL-2 in the treatment of advanced solid neoplasms, whereas there are no data about MLT influence on IL-2 activity in hematologic malignancies. The aim of the present study was to evaluate the efficacy and tolerability of a neuroimmunotherapeutic combination of low-dose IL-2 plus MLT in advanced hematologic malignancies which did not respond to previous standard therapies. The study included 12 evaluable patients. Tumor histotypes were as follows: non-Hodgkin's lymphoma (NHL) 6; Hodgkin's disease (HD), 2; multiple myeloma, 2; acute myelogenous leukemia (ALM), 1 and chronic myelomonocytic leukemia (CMML), 1. IL-2 was injected subcutaneously at a dose of 3 million IU/day for 6 days per week for 4 weeks, corresponding to one cycle. MLT was given orally at 20 mg/day in the evening, without interruption. In non-progressing patients, a second IL-2 cycle was planned after a 3 week-rest period. A partial response was achieved in one patient with multiple myeloma. Stable disease occurred in 7 other patients (NHL, 3; HD, 1; AML, 1; CLLM, 1; multiple myeloma, 1), whereas the other 4 patients progressed. Therefore, lack of progression was obtained in 8 out of 12 (67%) patients, with a median duration of 21+ months (14-30+ months). The treatment was well tolerated in all patients. These preliminary results would suggest that the concomitant administration of low-dose IL-2 plus the pineal hormone MLT may prolong the survival time in untreatable advanced hematologic malignancies, with results comparable to those previously reported using a more toxic immunotherapy, consisting of high-dose IL-2 alone.

Neuro Endocrinol Lett. 2001;22(1):45-7.

Anti-angiogenic activity of melatonin in advanced cancer patients.

Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni GJ.

OBJECTIVES: The anticancer activity of the indole melatonin has been explained to be due to its immunomodulatory, anti-prolferative and anti-oxidant effects, whereas at present no data are available about its possible influence on the angiogenesis, which has been shown to be one of the main biological mechanisms responsible for tumor dissemination. Vascular endothelial growth factor (VEGF) is the most active angiogenic factor, and the evidence of abnormally high blood levels or VEGF has been proven to be associated with poor prognosis in cancer patients. To investigate the influence of melatonin on angiogenesis, in this preliminary study we have evaluated the effects of melatonin therapy on VEGF blood levels in advanced cancer patients. MATERIAL & METHODS: The study included 20 metastatic patients, who progressed on previous conventional antitumor therapies and for whom no other effective treatment was available. Melatonin was given orally at 20 mg/day in the evening for at least 2 months. Serum levels of VEGF were measured by an enzyme immunoassay on venous blood samples collected at 15-day intervals. RESULTS: The clinical response consisted of minor response (MR) in 2, stable disease (SD) in 6 and progressive disease (PD) in the remaining 12 patients. VEGF mean levels decreased on therapy, without, however, statistical differences with respect to the pre-treatment values. In contrast, by evaluating changes in VEGF levels in relation to the clinical response, non-progressing patients (MR + SD) showed a significant decline in VEGF mean concentrations, whereas no effect was achieved in progressing patients. CONCLUSIONS: This study, by showing that melatonin-induced control or the neoplastic growth is associated with a decline in VEGF secretion, would suggest that the pineal hormone may control tumor growth at least in part by acting as a natural anti-angiogenic molecule, with a following opposition or angiogenesis-dependent cancer proliferation.

J Pineal Res. 1992 Apr;12(3):97-104.

Interaction of melatonin with human lymphocytes: evidence for binding sites coupled to potentiation of cyclic AMP stimulated by vasoactive intestinal peptide and activation of cyclic GMP.

Lopez-Gonzalez MA, Calvo JR, Osuna C, Guerrero JM.

Melatonin binding sites were characterized in human blood lymphocytes. The specific binding 2-[125I]iodo-melatonin ([125I]MEL) to human lymphocytes was dependent on time and temperature, stability, saturation, and reversibility. Moreover, guanine nucleotides decreased the specific binding of [125I]MEL to crude membranes of human lymphocytes, suggesting the coupling of these binding sites to a guanosine nucleotide binding regulatory protein(s). In competition studies, the specific binding of [125I]MEL to lymphocytes was inhibited by increasing concentrations of native melatonin. Scatchard analysis showed that data were compatible with the existence of two classes of binding sites: a high-affinity site with a Kd of 5.20 +/- 0.79 nM and a binding capacity of 50.6 +/- 11.0 fmol/10(7) cells, and a low-affinity site with a Kd of 208.5 +/- 50.2 nM and a binding capacity of 2691 +/- 265 fmol/10(7) cells. However, concentration-dependent binding of [125I]MEL to lymphocytes was saturable and resulted in a linear Scatchard plot, suggesting binding to a single class of binding sites. The Kd for the single site was 1.02 +/- 0.34 nM with a binding capacity of 10.1 +/- 1.6 fmol/10(7) cells. Their affinities closely correlated with the production of cyclic nucleotides, suggesting a physiological role for the melatonin binding sites. Thus, melatonin potentiated the effect of vasoactive intestinal peptide (VIP) on cyclic AMP production (ED50 = 1.9 nM) and stimulated cyclic GMP accumulation (ED50 = 125 nM). Results demonstrate the existence of two binding sites for [125I]MEL in human blood lymphocytes, with a high-affinity binding site coupled to the potentiation of the effect of VIP on cyclic AMP production and a low-affinity binding site coupled to activation of cyclic GMP production.

Front Neuroendocrinol. 2004 Sep-Dec;25(3-4):177-95.

Human pineal physiology and functional significance of melatonin.

Macchi MM, Bruce JN.

Descriptions of the pineal gland date back to antiquity, but its functions in humans are still poorly understood. In both diurnal and nocturnal vertebrates, its main product, the hormone melatonin, is synthesized and released in rhythmic fashion, during the dark portion of the day-night cycle. Melatonin production is controlled by an endogenous circadian timing system and is also suppressed by light. In lower vertebrates, the pineal gland is photosensitive, and is the site of a self-sustaining circadian clock. In mammals, including humans, the gland has lost direct photosensitivity, but responds to light via a multisynaptic pathway that includes a subset of retinal ganglion cells containing the newly discovered photopigment, melanopsin. The mammalian pineal also shows circadian oscillations, but these damp out within a few days in the absence of input from the primary circadian pacemaker in the suprachiasmatic nuclei (SCN). The duration of the nocturnal melatonin secretory episode increases with nighttime duration, thereby providing an internal calendar that regulates seasonal cycles in reproduction and other functions in photoperiodic species. Although humans are not considered photoperiodic, the occurrence of seasonal affective disorder (SAD) and its successful treatment with light suggest that they have retained some photoperiodic responsiveness. In humans, exogenous melatonin has a soporific effect, but only when administered during the day or early evening, when endogenous levels are low. Some types of primary insomnia have been attributed to diminished melatonin production, particularly in the elderly, but evidence of a causal link is still inconclusive. Melatonin administration also has mild hypothermic and hypotensive effects. A role for the pineal in human reproduction was initially hypothesized on the basis of clinical observations on the effects of pineal tumors on sexual development. More recent data showing an association between endogenous melatonin levels and the onset of puberty, as well as observations of elevated melatonin levels in both men and women with hypogonadism and/or infertility are consistent with such a hypothesis, but a regulatory role of melatonin has yet to be established conclusively. A rapidly expanding literature attests to the involvement of melatonin in immune function, with high levels promoting and low levels suppressing a number of immune system parameters. The detection of melatonin receptors in various lymphoid organs and in lymphocytes suggests multiple mechanisms of action. Melatonin has been shown to be a powerful antioxidant, and has oncostatic properties as well, both direct and indirect, the latter mediated by its effects on reproductive hormones. Finally, there are reports of abnormal daily melatonin

profiles in a number of psychiatric and neurological disorders, but the significance of such abnormalities is far from clear.

Cell Mol Biol Res. 1995;41(5):391-5.

Melatonin immunoreactivity in the photosynthetic prokaryote Rhodospirillum rubrum: implications for an ancient antioxidant system.

Manchester LC, Poeggeler B, Alvares FL, Ogden GB, Reiter RJ.

Rhodospirillum rubrum is a spiral anoxygenic photosynthetic bacterium that can exist under either aerobic or anaerobic conditions. The organism thrives in the presence of light or complete darkness and represents one of the oldest species of living organisms, possibly 2-3.5 billion years old. The success of this prokaryotic species may be attributed to the evolution of certain indole compounds that offer protection against life-threatening oxygen radicals produced by an evolutionary harsh environment. Melatonin, N-acetyl-5-methoxytryptamine, is an indolic highly conserved molecule that exists in protists, plants, and animals. This study was undertaken to determine the presence of an immunoreactive melatonin in the kingdom Monera and particularly in the photosynthetic bacterium, R. rubrum, under conditions of prolonged darkness or prolonged light. Immunoreactive melatonin was measured during both the extended day and extended night. Significantly more melatonin was observed during the scotophase than the photophase. This study marks the first demonstration of melatonin in a bacterium. The high level of melatonin observed in bacteria may provide on-site protection of bacterial DNA against free radical attack.

J Pineal Res. 2001 May;30(4):220-6.

Melatonin inhibits PHA-stimulated chicken lymphocyte proliferation in vitro.

Markowska M, Waloch M, Skwarlo-Sonta K.

Many studies have shown that melatonin plays a fundamental role in neuroimmunomodulation. There are known differences between mammals and birds in immunomodulatory function of melatonin exerted in vivo. In present study the effect of exogenous melatonin on chicken lymphoid cell proliferation in vitro was examined. Melatonin alone (10(-10)M-10(-5)M) did not exert any effect on the proliferation of the chicken thymocytes, splenocytes and lymphocytes from the bursa of Fabricius. On the other hand, melatonin addition strongly inhibited the proliferation of PHA-activated thymocytes and splenocytes from young chickens. The effect of melatonin was hormone- and mitogen-(PHA) dose-dependent; the most pronounced effect was obtained at low PHA and melatonin concentrations i.e., 1.6-3.125 microg/100 microl of PHA and 10(-10) M-10(-7) M melatonin, respectively. Prior immunization of chickens with sheep red blood cells (SRBC) inhibited the proliferative response of splenocytes to the same dose of PHA and, consequently, the effect of melatonin addition was not observed. The results reported demonstrate a direct inhibitory effect of melatonin on in vitro activated chicken lymphocytes.

J Immunol. 1994 Sep 15;153(6):2671-80.

Activation of human monocytes by the pineal hormone melatonin.

Morrey KM, McLachlan JA, Serkin CD, Bakouche O.

To determine the effects of the pineal hormone melatonin on human monocytes, human monocytes were activated by different concentrations of melatonin. Above the activation threshold of 5 x 10(-11) M, melatonin was able to induce the cytotoxicity of human monocytes, the secretion of IL-1, and the production of reactive oxygen intermediates. Melatonin and LPS seemed to have a synergistic effect on human monocyte activation. Indeed, below their respective monocyte activation threshold (5 x 10(-11) M and 0.625 ng/ml), melatonin (10(-12) M) in association with LPS (0.2 ng/ml) was able to induce cytotoxicity, IL-1 secretion, and reactive oxygen intermediates production. Melatonin alone at 10(-12) M or LPS alone at 0.2 ng/ml did not activate monocytes. Furthermore, melatonin was able to prime the monocytes for a subsequent activation by LPS. When monocytes were activated by LPS (0.25 ng/ml) at the time that they were plated and then activated by melatonin (10(-12) M) 8 h later, no IL-1 secretion and no cytotoxicity were detected. However, when the cells were first activated by melatonin (10(-12) M), and then 8 h later by LPS (0.25 ng/ml), IL-1 secretion and monocyte cytotoxicity were observed. Above its monocyte activation threshold, melatonin induces both cell-associated IL-1 alpha and IL-1 beta activities. Below this activation threshold, i.e., at 10(-12) M, melatonin does not induce the cell-associated IL-1 alpha and IL-1 beta activities, but does induce the mRNA for both IL-1 (alpha and beta). It seems that melatonin activates monocytes through protein kinase C. These data suggest that melatonin activates monocytes and induces their cytotoxic properties, along with the IL-1 secretion.

Cancer. 1994 Jun 15;73(12):3015-9.

Modulation of human lymphoblastoid interferon activity by melatonin in metastatic renal cell carcinoma. A phase II study.

Neri B, Fiorelli C, Moroni F, Nicita G, Paoletti MC, Ponchietti R, Raugei A, Santoni G, Trippitelli A, Grechi G.

BACKGROUND. Numerous attempts to identify active cytotoxic agents for the treatment of metastatic renal cell carcinoma (RCC) have proved disappointing. However, several recent developments in biologic therapy of neoplastic disease have substantially improved the prospects for the treatment of advanced RCC. Melatonin (MLT), a hormone regulated by the pineal gland, has been shown to act on the immune system by causing the release of cytokines from activated T-cell populations. METHODS. A series of 22 patients with documented progressing RCC entered a trial in which the authors studied the effect of a long term regimen (12 months) with human lymphoblastoid interferon (IFN), 3 mega units (MU) intramuscularly 3 times per week, and MLT, 10 mg orally every day. RESULTS. Twenty-one patients were evaluable for response and toxicity. There were seven remissions (33%): three complete, involving lung and soft tissue and four partial, with a median duration at the time of this writing of 16 months. Nine patients achieved stable disease, and five progressed. General toxicity was mild. Fever, chills, arthralgias, and myalgias occurred rarely. Leukopenia and hepatic enzyme elevation were modest and always reversible. CONCLUSIONS. Response rate and toxic effects observed during this study warrant additional randomized studies to define the role of MLT's concomitant administration in the clinical response to IFN in metastatic RCC.

J Pineal Res. 1997 May;22(4):184-202.

The validity of melatonin as an oncostatic agent.

Panzer A, Viljoen M.

The validity of melatonin as a prominent, naturally occurring oncostatic agent is examined in terms of its putative oncostatic mechanism of action, the correlation between melatonin levels and neoplastic activity, and the outcome of therapeutically administered melatonin in clinical trials. Melatonin's mechanism of action is summarized in a brief analysis of its actions at the cellular level, its antioxidative functions, and its indirect immunostimulatory effects. The difficulties of interpreting melatonin levels as a diagnostic or prognostic aid in cancer is illustrated by referral to breast cancer, the most frequently studied neoplasm in trials regarding melatonin. Trials in which melatonin was used therapeutically are reviewed, i.e., early studies using melatonin alone, trials of melatonin in combination with interleukin-2, and controlled studies comparing routine therapy to therapy in combination with melatonin. A table compiling the studies in which melatonin was used in the treatment of cancer in humans is presented according to the type of neoplasm. Melatonin's suitability in combination chemotherapy, where it augments the anticancer effect of other chemotherapeutic drugs while decreasing some of the toxic side effects, is described. Based on the evidence derived from melatonin's antiproliferative, antioxidative, and immunostimulatory mechanisms of action, from its abnormal levels in cancer patients and from clinical trials in which melatonin was administered, it is concluded that melatonin could indeed be considered a physiological anticancer substance. Further well-controlled trials should, however, be performed in order to find the link between its observed effects and the underlying mechanisms of action and to define its significance as a therapeutic oncostatic agent.

Neuro Endocrinol Lett. 2002 Apr;23 Suppl 1:24-9.

Oncostatic action of melatonin: facts and question marks.

Pawlikowski M, Winczyk K, Karasek M.

The paper presents the data concerning the in vivo effects of melatonin on experimentally-induced tumors in animals and the in vitro effects on animal and human tumor cells. The majority of experimental tumors responded to the melatonin treatment with growth inhibition. However, some negative or opposite results (i.e. stimulation of tumor instead of inhibition) were also reported. Some of the negative results can be attributed to the improper timing of melatonin administration. Melatonin was also shown to inhibit the growth of several animal and human tumor cell lines in vitro. On the basis of these experiments, a hypothesis of the oncostatic action of melatonin was put forward. The mechanism of the postulated action is complex and probably includes: 1) modulation of the endocrine system; 2) modulation of the immune system; 3) the direct oncostatic action of melatonin on tumor cells. The latter includes the recently discovered anti-oxidative action which probably plays an important role in the countering the DNA damage during the radiation challenge or the exposure to chemical carcinogens. It also includes the antiproliferative and pro-apoptotic effects exerted via melatonin receptors expressed by tumor cells. The involvement of the membrane melatonin receptors is mainly assumed. However, the recent data from our and other laboratories suggest also the involvement of RZR/ROR receptors (the putative melatonin nuclear receptors) in both melatonin-induced proliferation inhibition and apoptosis.

Endocr Res. 1996 Feb;22(1):77-94.

Melatonin and 2[125I]iodomelatonin binding sites in the human colon.

Poon AM, Mak AS, Luk HT.

2[125I]Iodomelatonin binding sites were identified in the mucosa of the human colon from Chinese patients with carcinoma of the rectum or colon using biochemical receptor assay and autoradiography. Melatonin in the colonic mucosa/submucosa and muscle layers were quantitated by radioimmunoassay. The binding of 2[125I]iodomelatonin to the membrane preparations of the human colonic mucosa/submucosa was stable, saturable, reversible and of high affinity. Rosenthal analysis from saturation studies performed at 21 degrees C yielded an equilibrium dissociation constant (Kd) of 61.7 +/- 4.48 pmol/L (n = 3) and maximum number of binding sites (B(max)) of 1.65 +/- 0.51 fmol/mg protein (n = 3). The linearity of the Rosenthal plots and unity of the Hill coefficient suggested that 2[125I]iodomelatonin was bound to a single class of binding sites. The radioligand binding was displaced by 2-iodomelatonin (Ki = 0.02 nmol/L), melatonin (0.65 nmol/L), 6-chloromelatonin (Ki = 5.33 nmol/L), 6-hydroxymelatonin (Ki = 33.8 nmol/L) and N-acetylserotonin (Ki = 122 nmol/L). The characteristic of the binding sites were similar to those reported in the jejunum of duck, chicken, and human but of higher affinity than those in the mouse colon. Autoradiography localizes the binding to the mucosa of the human colon. Radioimmunoassay revealed a melatonin concentration of 467 +/- 99 pg/g wet tissue of human colon (n = 6). Our findings suggest that melatonin may influence the human colonic functions through interaction with its receptors in the mucosa.

Life Sci. 1998;63(19):1701-13.

Melatonin decreases bone marrow and lymphatic toxicity of adriamycin in mice bearing TLX5 lymphoma.

Rapozzi V, Zorzet S, Comelli M, Mavelli I, Perissin L, Giraldi T.

When CBA male mice bearing TLX5 lymphoma were treated in the evening with a single i.v. dose of adriamycin (20-40 mg/Kg), the administration of a single pharmacological dose of melatonin (10 mg/kg s.c.) 1 hr earlier reduced the acute mortality from 10/24 to 2/24. The increase in survival time caused by adriamycin over drug untreated controls was not reduced by melatonin. The administration of melatonin alone did not cause any antitumor or evident toxic effect. Melatonin also attenuated the reduction caused by adriamycin in the number of bone marrow GM-CFU, and of CD3+, CD4+ and CD8+ splenic T-lymphocyte subsets. Reduced and total glutathione levels were decreased in the bone marrow and in the liver cells of the animals treated with adriamycin, and were significantly restored by melatonin. Moreover, lipid peroxidation by adriamycin was reduced by melatonin, as indicated by malondialdehyde measurement in the liver of the treated animals. These data indicate that the protective effects of melatonin against the host toxicity of the prooxidant antitumor drug, adriamycin, might be attributed at least partially to its antioxidant properties. These findings appear of interest in relation to the physiological rhythmic levels of endogenous melatonin and to the chronotoxicology of anthracyclines.

Ann N Y Acad Sci. 2002 Apr;959:238-50.

Melatonin reduces oxidant damage and promotes mitochondrial respiration: implications for aging.

Reiter RJ, Tan DX, Manchester LC, El-Sawi MR.

Melatonin has a number of properties as a consequence of which it could be beneficial to animals as they age. Of particular interest are its ubiquitous actions as a direct and indirect antioxidant and free radical scavenger. Besides directly detoxifying a variety of reactive oxygen and reactive nitrogen species, at least one product that is formed as a result of these interactions is also a potent free radical scavenger. Thus, the product that is formed when melatonin detoxifies hydrogen peroxide, that is, N1-acetyl-N2-formyl-5-methoxykynuramine is an efficient scavenger, at least equivalent to melatonin itself. This antioxidant cascade increases the ability of melatonin to resist oxidative damage. Other actions of melatonin, such as stimulation of antioxidative enzymes also improves its status as an antioxidant. Finally, recent observations documenting melatonin's ability to stimulate electron transport and ATP production in the inner-mitochondrial membrane also has relevance for melatonin as an agent that could alter processes of aging. These findings, coupled with diminished melatonin production in advanced age, has prompted scientists to consider melatonin in the context of aging. As of this writing there is no definitive evidence to prove that melatonin alters the rate of aging, although data relating to melatonin deferring some age-related degenerative conditions is accumulating rapidly.

Proc Natl Acad Sci U S A. 1995 Sep 12;92(19):8734-8.

Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor.

Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF.

A G protein-coupled receptor for the pineal hormone melatonin was recently cloned from mammals and designated the Mel1a melatonin receptor. We now report the cloning of a second G protein-coupled melatonin receptor from humans and designate it the Mel1b melatonin receptor. The Mel1b receptor cDNA encodes a protein of 362 amino acids that is 60% identical at the amino acid level to the human Mel1a receptor. Transient expression of the Mel1b receptor in COS-1 cells results in high-affinity 2-[125I]iodomelatonin binding (Kd = 160 +/- 30 pM). In addition, the rank order of inhibition of specific 2-[125I]iodomelatonin binding by eight ligands is similar to that exhibited by the Mel1a melatonin receptor. Functional studies of NIH 3T3 cells stably expressing the Mel1b melatonin receptor indicate that it is coupled to inhibition of adenylyl cyclase. Comparative reverse transcription PCR shows that the Mel1b melatonin receptor is expressed in retina and, to a lesser extent, brain. PCR analysis of human-rodent somatic cell hybrids maps the Mel1b receptor gene (MTNR1B) to human chromosome 11q21-22. The Mel1b melatonin receptor may mediate the reported actions of melatonin in retina and participate in some of the neurobiological effects of melatonin in mammals.

Neuro Endocrinol Lett. 2002 Apr;23 Suppl 1:61-6.

Melatonin in immunity: comparative aspects.

Skwarlo-Sonta K.

Pineal gland, by the diurnal rhythm of synthesis and release of its principal hormone, melatonin (MEL), is involved in reciprocal relationships between neuroendocrine and immune systems, responsible for keeping internal homeostasis in vertebrate animals. In this paper the experimental data, indicating that both strategic (developmental, thus antigen independent) and emergency (evoked by antigenic activation of the mature immune system) levels of interactions between pineal gland and immune system, operate in mammals and birds, are reviewed. The cells and organs of immune system using membrane receptors as well as nuclear orphan receptors perceive MEL message. Effects exerted by MEL on immune parameters are different, and depend on several factors, including dose and way of MEL application, species, sex, age of animal, its immune system maturation, way of immune system activation, and parameter examined, as well as the season, circadian rhythm of both immunity and pineal gland function, stressful conditions, accompanying experimental procedure, etc. In turn, lymphoid organ-derived hormones and cytokines, soluble factors secreted by activated immune cells act as messages understood by the pineal gland, closing the regulatory loop of the bi-directional functional connections between both systems.

Genomics. 1995 May 20;27(2):355-7.

Mapping of the gene for the Mel1a-melatonin receptor to human chromosome 4 (MTNR1A) and mouse chromosome 8 (Mtnr1a).

Slaugenhaupt SA, Roca AL, Liebert CB, Altherr MR, Gusella JF, Reppert SM.

The pineal hormone melatonin elicits potent circadian and reproductive effects in mammals. We report the chromosomal location of the gene for the Mel1a-melatonin receptor that likely mediates these circadian and reproductive actions. PCR analysis of human-rodent somatic cell hybrids showed that the receptor gene (MTNR1A) maps to human chromosome 4q35.1. An interspecific backcross analysis revealed that the mouse gene (Mtnr1a) maps to the proximal portion of chromosome 8. These loci may be involved in genetically based circadian and neuroendocrine disorders.

J Pineal Res. 2004 Sep;37(2):98-106.

Melatonin stimulates calmodulin phosphorylation by protein kinase C.

Soto-Vega E, Meza I, Ramirez-Rodriguez G, Benitez-King G.

Calmodulin (CaM)-dependent processes can be modulated by the availability of Ca(+2), the subcellular distribution of both CaM and its target proteins, CaM antagonism, and post-translational modifications such as CaM phosphorylation. Melatonin, the pineal secretory product synthesized during the dark phase of the photoperiod is an endogenous CaM antagonist. This indolamine causes CaM subcellular redistribution in epithelial MDCK and MCF-7 cells, and selectively activates protein kinase C alpha (PKC alpha) in neuronal N1E-115 cells. In the present work we have characterized the phosphorylation of CaM mediated by PKC alpha and its stimulation by melatonin in an in vitro reconstituted enzyme system. Additionally, the participation of MAPK and ERKs, downstream kinases of the PKC signaling pathway, was explored utilizing MDCK cell extracts as source of these kinases. Phosphorylation of CaM was characterized in the whole cells by MDCK cell metabolic labeling with [(32)P]-orthoposhospate, and CaM separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, as well as by immunocolocalization of phosphorylated threonine/serine residues and CaM in cultured cells incubated with melatonin. Our results show that melatonin increased CaM phosphorylation by PKC alpha with an EC(50) of 10(-8) m in the presence of the phorbol ester, phorbol-12-myristate-13-acetate (PMA) in the in vitro reconstituted enzyme system. An increase in phosphorylated CaM was also observed in cells cultured with melatonin, or PMA for 2 hr, while, PKC, MAPK, or ERK inhibitors abolished CaM phosphorylation elicited by melatonin in MDCK cell extracts. Our data show that melatonin can stimulate phosphorylation of CaM by PKC alpha in the in vitro reconstituted system and suggest that in MDCK cells this phosphorylation is accomplished by PKC. Modification of CaM by melatonin can be another route to inhibit CaM interaction with its target enzymes.

Experientia. 1989 Oct 15;45(10):922-32.

Melatonin biosynthesis in the mammalian pineal gland.

Sugden D.

Rhythmic production of melatonin by the mammalian pineal occurs in response to noradrenergic stimulation which produces a cascade of biochemical events within the pinealocyte. In the rat, massive changes in NAT activity result from an increase in intracellular c-AMP levels produced by a synergistic interaction whereby an alpha 1 activation amplifies beta-adrenergic stimulation. The intracellular events mediating this effect are described. A major aspect of the temporal control of melatonin production is the programmed down-regulation of responses to noradrenergic stimulation once the initial surge of c-AMP is produced. Noradrenergic activation of the gland also influences other enzymic functions, including tryptophan hydroxylase and HIOMT activities, and produces a dramatic increase in intracellular c-GMP levels. Other neurotransmitters and neuropeptides, e.g. VIP, may also influence pineal function and comparisons are made between the rat, the subject of the bulk of experimental studies, and other species.

Pigment Cell Res. 2004 Oct;17(5):454-60.

Melatonin, melatonin receptors and melanophores: a moving story.

Sugden D, Davidson K, Hough KA, Teh MT.

Melatonin (5-methoxy N-acetyltryptamine) is a hormone synthesized and released from the pineal gland at night, which acts on specific high affinity G-protein coupled receptors to regulate various aspects of physiology and behaviour, including circadian and seasonal responses, and some retinal, cardiovascular and immunological functions. In amphibians, such as Xenopus laevis, another role of melatonin is in the control of skin coloration through an action on melanin-containing pigment granules (melanosomes) in melanophores. In these cells, very low concentrations of melatonin activate the Mel(1c) receptor subtype triggering movement of granules toward the cell centre thus lightening skin colour. Mel(1c) receptor activation reduces intracellular cAMP via a pertussis toxin-sensitive inhibitory G-protein (Gi), but how this and other intracellular signals regulate pigment movement is not yet fully understood. However, melanophores have proven an excellent model for the study of the molecular mechanisms which coordinate intracellular transport. Melanosome transport is reversible and involves both actin- (myosin V) and microtubule-dependent (kinesin II and dynein) motors. Melanosomes retain both kinesin and dynein during anterograde and retrograde transport, but the myosin V motor seems to be recruited to melanosomes during dispersion, where it assists kinesin II in dominating dynein thus driving net dispersion. Recent work suggests an important role for dynactin in coordinating the activity of the opposing microtubule motors. The melanophore pigment aggregation response has also played a vital role in the ongoing effort to devise specific melatonin receptor antagonists. Much of what has been learnt about the parts of the melatonin molecule required for receptor binding and activation has come from detailed structure-activity data using novel melatonin ligands. Work aiming to devise ligands specific for the distinct melatonin receptor subtypes stands poised to deliver selective agonists and antagonists which will be valuable tools in understanding the role of this enigmatic hormone in health and disease.

Cancer Biother Radiopharm. 2001 Apr;16(2):171-7.

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.

Todisco M, Casaccia P, Rossi N.

PURPOSE: Somatostatin, prolactin, retinoids, melatonin and ACTH have been shown to influence the lymphatic growth, and the action of the cyclophosphamide in lymphoproliferative disorders is well known. This provided the rationale to conduct, in patients with low-grade non-Hodgkin's lymphomas (NHL), a phase II trial of a combined association of cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin and ACTH. PATIENTS AND METHODS: Twenty patients with a diagnosis of low-grade NHL, stage III or IV, were included in this study. Patients received for one month the following treatment: cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin, and ACTH. The therapy was continued for two additional months in patients with stable or responding disease. After three months, the responding patients continued the therapy for three months and more. RESULTS: Twenty patients were assessable for toxicity and response; 70% (14 of 20 patients; 95% confidence interval [CI], 50% to 90%) had a partial response; 20% (4 of 20) had stable disease, and 10% (2 of 20) progressed on therapy. Going on with the treatment, none of the 14 patients with partial response had a disease progression (average follow-up time of 21 months, range, 7 to 25), and 50% of these patients had a complete response; among 4 patients with stable disease, 25% (1 of 4) had a partial response and 75% (3 of 4) progressed on therapy (mean time to progression [TTP] 14.3 months, range, 7 to 21). The toxicity was very mild, the most common side effects being drowsiness, diarrhea and hyperglycemia. CONCLUSIONS: The association of cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin, and ACTH is well tolerated and effective in treatment of low-grade NHL at advanced stage.

J Pineal Res. 1992 Sep;13(2):60-5.

Binding sites for [3H]-melatonin in human platelets.

Vacas MI, Del Zar MM, Martinuzzo M, Cardinali DP.

A number of in vitro effects of melatonin on human platelets were revealed in previous studies. In order to examine whether high affinity binding sites for [3H]-melatonin were present in membrane preparations of human platelets, a rapid filtration procedure through Whatman GFB paper was employed. Maximal melatonin binding was attained within 3 hr at 0 degree C. Scatchard analysis indicated a single population of binding sites with a dissociation constant (Kd) = 4.1 +/- 0.5 nM and maximal number of binding sites (Bmax) = 24.2 +/- 1.9 fmol/mg protein (mean +/- SEM of five experiments). When various indole analogs were tested for their ability to inhibit [3H]-melatonin binding, the following Ki (nM) were obtained: 6-chloromelatonin (11.4), 2-iodomelatonin (22.0), melatonin (24.7), 5-methoxytryptophol (49.9), N-acetylserotonin (68.9), 6-hydroxymelatonin (78.2), 5-methoxytryptamine (184). Serotonin was a potent inhibitor of [3H]-melatonin binding with a Ki = 20.6 nM. Except for 2-methylserotonin and alpha-methylserotonin, a number of serotonin agonists and antagonists tested did not affect melatonin binding to platelet membranes. Binding experiments carried out at either 0800 or 2000 did not reveal time-dependent differences in Kd or Bmax. The results suggest that high affinity melatonin acceptors are present in human platelets.

Clin Biochem. 1992 Apr;25(2):125-7.

Putative melatonin receptor in human spermatozoa.

van Vuuren RJ, Pitout MJ, van Aswegen CH, Theron JJ.

Melatonin is present in human semen, and may affect sperm motility. The presence of melatonin receptors on spermatozoa has not yet been reported. Detection of melatonin-binding sites may be limited because of the masking of such sites by sialic acid. Spermatozoa were obtained from eligible human donors, incubated with neuraminidase to remove sialic acid residues, and saturation binding assays were carried out using 2-125I-melatonin as a receptor probe. Consistent 125I-melatonin binding could only be obtained after spermatozoa were treated with neuraminidase. Scatchard analysis revealed a low-affinity binding site (ML-2) with a Kd value of 127 +/- 6 nM and a Bmax of 25 +/- 4.5 fmol/mg protein. These results present evidence of melatonin-binding sites in spermatozoa. Sialic acid possibly regulates the binding of melatonin to these sites.

Int J Radiat Oncol Biol Phys. 2004 Jul 1;59(3):639-53.

Melatonin as a radioprotective agent: a review.

Vijayalaxmi, Reiter RJ, Tan DX, Herman TS, Thomas CR Jr.

Melatonin (N-acetyl-5-methoxytryptamine), the chief secretory product of the pineal gland in the brain, is well known for its functional versatility. In hundreds of investigations, melatonin has been documented as a direct free radical scavenger and an indirect antioxidant, as well as an important immunomodulatory agent. The radical scavenging ability of melatonin is believed to work via electron donation to detoxify a variety of reactive oxygen and nitrogen species, including the highly toxic hydroxyl radical. It has long been recognized that the damaging effects of ionizing radiation are brought about by both direct and indirect mechanisms. The direct action produces disruption of sensitive molecules in the cells, whereas the indirect effects (approximately 70%) result from its interaction with water molecules, which results in the production of highly reactive free radicals such as *OH, *H, and e(aq)- and their subsequent action on subcellular structures. The hydroxyl radical scavenging ability of melatonin was used as a rationale to determine its radioprotective efficiency. Indeed, the results from many in vitro and in vivo investigations have confirmed that melatonin protects mammalian cells from the toxic effects of ionizing radiation. Furthermore, several clinical reports indicate that melatonin administration, either alone or in combination with traditional radiotherapy, results in a favorable efficacy:toxicity ratio during the treatment of human cancers. This article reviews the literature from laboratory investigations that document the ability of melatonin to scavenge a variety of free radicals (including the hydroxyl radical induced by ionizing radiation) and summarizes the evidence that should be used to design larger translational research-based clinical trials using melatonin as a radioprotector and also in cancer radiotherapy. The potential use of melatonin for protecting individuals from radiation terrorism is also considered.

J Clin Endocrinol Metab. 1995 May;80(5):1747-9.

Melatonin receptors on human granulosa cell membranes.

Yie SM, Niles LP, Younglai EV.

Putative melatonin binding sites were detected in the membrane fraction of gonadotropin-stimulated human granulosa cells using the melatonin analogue 2-[125I]-iodomelatonin (125I-IML). Saturation studies and Scatchard analysis revealed the presence of a major binding site with a Kd of 99 pM. Guanosine triphosphate shifted the receptor affinity to 380 pM. In competition studies, the rank order of potency of indoles for inhibition of 125I-IML binding at these sites was typical of melatonin receptors: 2-iodomelatonin > melatonin > N-acetylserotonin > 5-methoxytryptamine > serotonin. Culture of cells for 7 days in vitro increased receptor density but not the affinity. These findings strongly suggest that melatonin found in follicular fluid may have a physiological role.