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The impact of agomelatine and melatonin on human colorectal cancer: An in vitro investigation

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Abstract

Aim: Colorectal cancer (CRC) impacts almost one million people worldwide each year, with 500,000 deaths annually. In our country, CRC ranks as the third most prevalent cancer in women and the fourth in men. Melatonin, a hormone with well-established antioxidant and anti-cancer properties, is produced by the pineal gland. Agomelatine, used to antidepressant therapies, also acts on melatonin receptors and may offer therapeutic benefits in cancer treatment. This study investigates the effects of melatonin and agomelatine on the viability of human colorectal cancer cell lines (HCT-116 and Caco-2).

Materials and Methods: HCT-116 and Caco-2 cell lines were treated with melatonin and Agomelatine, both dissolved in 96% ethanol, at doses of 0.1, 1, 5, and 10 mM for a duration of 24 hours. Cell viability was measured using 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromide (MTT) assay. Statistical analyses were performed using IBM SPSS Statistics 24.0, applying Bonferroni correction and the Mann-Whitney U-test, with significance set at $p<0.05$. The half-maximal inhibitory concentration ($LogIC_{50}$) was calculated from the MTT assay results.

Results: All tested concentrations of melatonin and agomelatine significantly reduced cell viability in both HCT-116 and Caco-2 cell lines ($p<0.05$).

Conclusion: The study concludes that agomelatine and melatonin have substantial cytotoxic and anti-tumor properties against human colorectal cancer cells.

Introduction

Colorectal cancer (CRC) is the most common cancer worldwide and ranks among the top three in cancer-related mortality [1]. Current CRC treatments primarily involve chemotherapeutic agents, which may be used alone or in combination [2-4]. However, these treatments often come with significant drawbacks, such as severe side effects and the potential for developing resistance [5]. This underscores the urgent need for novel therapeutic approaches to enhance treatment efficacy.

Circadian rhythms, which are roughly 24-hour biological cycles, govern essential physiological processes such as the sleep-wake cycle, reproductive functions, and immune responses. Disruptions in these rhythms have been linked to the onset and progression of various cancers [6, 7]. Moreover, circadian rhythms can influence the effectiveness and tolerability of treatments like radiotherapy and chemotherapy [8].

Research into circadian rhythms has increased significantly, driven by the potential to prevent cancer and improve treatment strategies, thereby enhancing the quality of life for cancer patients [8, 9]. Melatonin, a hormone predominantly secreted by the pineal gland but also found in other tissues, plays a crucial role in regulating these rhythms [10]. It has shown promising anticancer effects across various types of cancer, including CRC, where it has been found to inhibit tumor growth, reduce proliferation, and induce apoptosis in both in vitro [11, 12] and in vivo models [13, 14]. Melatonin acts mainly through its G-protein coupled receptors, MT_1 and MT_2 [10]. Notably, lower levels of these receptors have been observed in CRC tumor samples compared to normal mucosa [15], suggesting that targeting these receptors with non-selective agonists could offer new therapeutic possibilities for CRC.

Agomelatine, a melatonin analog employed as an antidepressant, exhibits potent agonistic action at MT_1 and MT_2 receptors, as well as antagonistic activity at the serotonin $5\text{-}HT_{2C}$ receptor [16]. It has been shown to synchronize circadian rhythms, potentially through interactions with MT_1 and MT_2 receptors [17, 18]. Given the role of sero-

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tonin in cancer progression, including CRC, and the potential of serotonin receptor inhibitors as treatments [19], agomelatine's effects on circadian rhythms and receptor interactions make it a promising candidate for CRC therapy.

This study investigates the effects of melatonin and agomelatine on the viability of human colorectal cancer cell lines (HCT-116 and Caco-2).

Materials and Methods

Cell cultures

In this study, HCT-116 and Coca-2 cell lines were employed to investigate the effects of melatonin and agomelatine on cancer cell viability. The cell lines were obtained from ATCC and cultured in 25 cm^2 and 75 cm^2 flasks with RPMI-1640 medium (Sigma-Aldrich, USA), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The medium was refreshed every two days. The cells were maintained in a 5% CO_2 incubator (Panasonic, Japan) at 37 °C with a humidified atmosphere. Upon reaching confluence, the cells were detached using a trypsin-EDTA solution (Sigma-Aldrich, USA). Cell viability was assessed with 0.4% trypan blue staining, and experiments were initiated when cell viability was above 90%.

Preparation of melatonin and agomelatine

Melatonin (Sigma-Aldrich, USA) and agomelatine (Sigma-Aldrich, USA) were dissolved in ethyl alcohol (ETOH; Merck, Germany). To prepare working solutions, we created 0.1, 1, 5, and 10 mM concentrations of each compound in RPMI-1640 medium, following the evaporation of the ETOH.

MTT assay

To evaluate cytotoxicity, cells were counted using a hemocytometer after detachment with trypsin-EDTA. They were then seeded into 96-well plates at a density of $1.5x10^4$ cells per well in 200 µL of RPMI-1640 medium. After allowing cells to adhere at 37 °C for 24 hours in a CO_2 incubator, the wells were treated with 0.1, 1, 5, and 10 mM concentrations of melatonin and agomelatine. These doses were determined based on $LogIC_{50}$ values. The cells were incubated for an additional 24 hours. Following treatment, 0.5 mg/mL of MTT solution in sterile PBS was added to the wells, and the plates were incubated for 3 hours. The reaction was stopped by adding dimethyl sulfoxide (DMSO), and absorbance was measured at 550 nm using a spectrophotometer (Synergy HTX, USA). Absorbance values from control wells, representing 100% viability, were used as a reference. The viability of treated cells was calculated relative to the control. The MTT assay was performed in triplicate on 10 separate occasions, and the logarithmic half-maximal inhibitory concentration ($LogIC_{50}$) values were determined using GraphPad Prism 6 software.

Statistical analysis

Data analysis was carried out using IBM SPSS Statistics 24.0. Normal distribution of data was assessed using the Shapiro-Wilk test. The numbers of cell ($1.5x10^4$ cells per well in 200 µL) to be used in the experiments; 8% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power=0.80) were determined by power analysis. Comparisons between groups were performed with the Kruskal-Wallis H test for quantitative variables. For significant differences, pairwise comparisons were made using the Mann-Whitney U test with Bonferroni correction. A p-value of <0.05 was considered statistically significant. $LogIC_{50}$ values for melatonin and agomelatine were calculated using GraphPad Prism 6 based on the MTT assay results.

Results

The effects of melatonin and agomelatine on cell viability were assessed after 24 hours of incubation with HCT-116 cells and are depicted in Figure 1A and Figure 1B, respectively. Both melatonin and agomelatine, at concentrations of 0.1, 1, 5, and 10 mM, statistically significantly reduced cell viability in HCT-116 cells ($p<0.05$). Specifically, all tested concentrations of melatonin led to a notable decrease in cell viability, while agomelatine also exhibited a statistically significant reduction in viability across the

Table 1. $LogIC_{50}$ (mM) concentrations of melatonin and agomelatinin calculated for HCT-116 and Caco-2 cells in GraphPad Prizm 6 program.

Cell type	Melatonin $LogIC_{50}$ (mM)	Agomelatinin $LogIC_{50}$ (mM)
HCT-116	0.28	0.17
Caco-2	-0.26	-0.62

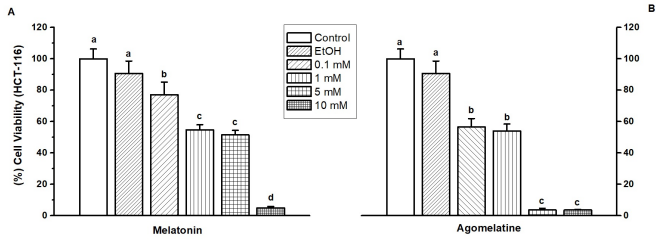

Figure 1. % changes in the viability of HCT-116 cells caused by melatonin (A) and agomelatinin (B) applied to the cell line for 24 hours (Different letters indicate the difference between two groups; a, b, c, d, $p<0.05$).

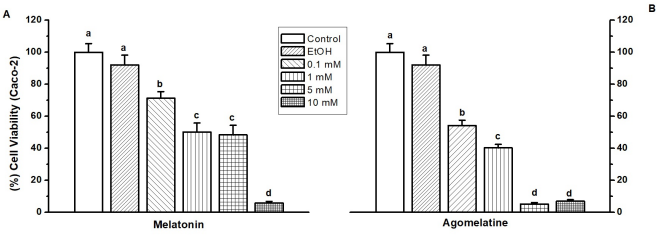

Figure 2. % changes in the viability of Caco-2 cells caused by melatonin (A) and agomelatinin (B) applied to the cell line for 24 hours (Different letters indicate the difference between two groups; a,b,c,d, $p<0.05$).

same concentrations ($p<0.05$).

Similarly, Figure 2A and Figure 2B illustrate the effects of melatonin and agomelatine on Caco-2 cells after 24 hours. In these experiments, all concentrations of both substances (0.1, 1, 5, and 10 mM) caused a statistically significant reduction in Caco-2 cell viability ($p<0.05$).

The $LogIC_{50}$ values for HCT-116 and Caco-2 cells, derived from MTT assays conducted after 24-hour treatments with melatonin and agomelatine, are summarized in Table 1. The $LogIC_{50}$ values suggest that melatonin was more effective at lower concentrations in Caco-2 cells, whereas agomelatine showed greater efficacy at lower concentrations in HCT-116 cells.

Discussion

Colorectal cancer, being the most frequently diagnosed malignancy globally, presents significant challenges due to high mortality and morbidity rates [21]. While drug therapies form the cornerstone of first-line chemotherapy for colorectal cancer, their effectiveness often diminishes in advanced stages [22]. Combination therapies, though beneficial, can lead to increased cytotoxicity and side effects [23]. Drug resistance further complicates treatment, highlighting the need for novel therapeutic approaches and targets.

This study explores the potential of agomelatine, a melatonin receptor agonist, in treating colorectal cancer. Our findings indicate that agomelatine induces cell death by inhibiting proliferation in HCT-116 and Caco-2 cell models. The anticancer efficacy of melatonin has been extensively shown in diverse cancer cell lines [24-30]. For instance, melatonin has been reported to possess anti-carcinogenic properties without significantly impacting DNA synthesis or cell growth [31]. Additionally, Hong et al. demonstrated that melatonin activates cell death pathways in HCT-116 cells [32], while Wei et al. found that melatonin induces apoptosis in colorectal LoVo cancer cells through dephosphorylation [33]. These findings support the notion that melatonin could be a valuable treatment for colorectal cancer by regulating carcinogenicity and impeding disease progression [34]. Our study confirms that melatonin effectively reduces cell viability in both HCT-116 and Caco-2 cells (Figures 1 and 2).

Although research on melatonin's anticancer effects is extensive, studies on agomelatine, its agonist, are relatively limited. One study reported that both melatonin and agomelatine induce autophagy in A172 and U87-MG glioblastoma cells in a concentration- and time-dependent manner, an effect significantly reduced by luzindole, a melatonin receptor antagonist [35]. Moreno et al. suggested that agomelatine might be beneficial in treating drug-resistant colorectal cancer patients [36]. Our results show that agomelatine significantly decreases cell viability in HCT-116 and Caco-2 cells, similar to melatonin. $LogIC_{50}$ values indicate that agomelatine has comparable cytotoxic activity to melatonin.

In summary, this study demonstrates that melatonin significantly decreases the viability of HCT-116 and Caco-2 cells. Furthermore, agomelatine, as a melatonin receptor agonist, exhibits substantial cytotoxic effects on these cancer cells, comparable to melatonin. These results suggest that agomelatine could be an important candidate for colorectal cancer treatment. In the future, it may be important to further investigate the mechanistic connections between agomelatine and cancer at the cellular level.

Ethical approval

It is a cell study that does not require ethics committee approval.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2020;70(4):313. PMID: 30207593.
2. Van Cutsem E, Cervantes A, Nordlinger B, Arnold D, & ESMO Guidelines Working Group (2014). Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology, 25 Suppl 3, iii1–iii9.
3. Seymour MT, Maughan TS, Ledermann JA, et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet (London, England), Lancet 2007; 370(9582), 143–152.
4. Koopman M, Antonini NF, Douma J, et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet (London, England), Lancet. 2007; 370 :135–142.
5. Vodenkova S, Buchler T, Cervena K, et al. 5-Fluorouracil and Other Fluoropyrimidines in Colorectal Cancer: Past, Present and Future. Pharmacol. Ther. 2020, 206, 107447.
6. Pérez-Villa A, Echeverría-Garcés G, Ramos-Medina MJ, et al. Integrated multi-omics analysis reveals the molecular interplay between circadian clocks and cancer pathogenesis. Sci Rep. 2023; 30;13(1):14198.
7. Kelleher FC, Rao A, Maguire A. Circadian molecular clocks and cancer. Cancer Lett. 2014; 342(1):9-18.
8. Lee Y, Field JM, Sehgal A. Circadian Rhythms, Disease and Chronotherapy. J Biol Rhythms. 2021; 36(6):503-531.
9. Kondratov R. Circadian clock and cancer therapy: an unexpected journey. Ann Med. 2014; 46(4):189-90.
10. Ekmekcioglu C. Expression and putative functions of melatonin receptors in malignant cells and tissues. Wien Med Wochenschr. 2014; 164(21-22):472-8.
11. Hamed AR, Yahya SMM, Nabih HK. Anti-drug resistance, anti-inflammation, and anti-proliferation activities mediated by melatonin in doxorubicin-resistant hepatocellular carcinoma: in vitro investigations. Naunyn Schmiedebergs Arch Pharmacol. 2023; 396(6):1117-1128..
12. Maroufi NF, Rashidi M, Vahedian V, et al. Effect of Apatinib plus melatonin on vasculogenic mimicry formation by cancer stem cells from breast cancer cell line. Breast Cancer. 2022; 29(2):260-273.
13. Tamarkin L, Cohen M, Roselle D, et al. Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res. 1981; 41(11 Pt 1):4432-6.
14. Anisimov VN, Popovich IG, Zabezhinski MA. Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal tumors induced by 1,2-dimethylhydrazine in rats. Carcinogenesis. 1997; 18(8):1549-53.
15. León J, Casado J, Carazo A, et al. Gender-Related Invasion Differences Associated with MRNA Expression Levels of Melatonin Membrane Receptors in Colorectal Cancer. Molecular carcinogenesis. 2012; 51(8), 608–618.
16. Sansone RA, Sansone LA. Agomelatine: a novel antidepressant. Innov Clin Neurosci. 2011;8(11):10-4.
17. Morley-Fletcher S, Mairesse J, Soumier A, et al. Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats. Psychopharmacology, 2011; 217(3): 301–313.
18. Popoli M. Agomelatine: innovative pharmacological approach in depression. CNS Drugs. 2009; 23: 27-34.

19. Sakita JY, Bader M, Santos ES, et al. Serotonin synthesis protects the mouse colonic crypt from DNA damage and colorectal tumorigenesis. J Pathol. 2019; 249(1):102-113.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55-63.
21. Dehghanzad M, Mohammadi M, Nejati M, et al. The potential therapeutic effect of melatonin in oxaliplatin combination therapy against chemoresistant colorectal cancer cells. Mol Biol Rep. 2024; 51(1):348.
22. Zhao Y, Wang C, Goel A. A combined treatment with melatonin and andrographis promotes autophagy and anticancer activity in colorectal cancer. Carcinogenesis 2022; 43(3):217–230.
23. Naderi Alizadeh M., Rashidi M., Muhammadnejad A., et al. Antitumor Effects of Umbelliprenin in a Mouse Model of Colorectal Cancer. Iranian journal of pharmaceutical research. 2018; 17(3): 976–985.
24. Sanchez-Hidalgo M, Guerrero JM, Villegas I, et al. Melatonin, a natural programmed cell death inducer in cancer. Curr Med Chem 2012; 19: 3805- 3821.
25. Zhang S, Qi Y, Zhang H, et al. Melatonin inhibits cell growth and migration, but promotes apoptosis in gastric cancer cell line, SGC7901. Biotech Histochem 2013; 88: 281-289.
26. Hermann R, Podhajsky S, Jungnickel S, Lerchl A. Potentiation of antiproliferative effects of tamoxifen and ethanol on mouse hepatoma cells by melatonin: Possible involvement of mitogen-activated protein kinase and induction of apoptosis. J Pineal Res 2002; 33: 8-13.
27. Martin V, Garcia-Santos G, Rodriguez-Blanco J, et al. Melatonin sensitizes human malignant glioma cells against TRAIL-induced cell death. Cancer Lett 2010; 287: 216-223.
28. Jung B, Ahmad N. Melatonin in cancer management: progress and promise. Cancer Res 2006; 66: 9789-9793.
29. Rodriguez C, Martin V, Herrera F, et al. Mechanisms involved in the pro-apoptotic effect of melatonin in cancer cells. Int J Mol Sci 2013; 14: 6597-6613.
30. Di Bella G, Mascia F, Gualano L, Di Bella L. Melatonin anticancer effects: review. Int J Mol Sci 2013; 14: 2410- 2430.
31. Farriol M, Venereo Y, Orta X, Castellanos JM, Segovia- Silvestre T. In vitro effects of melatonin on cell proliferation in a colon adenocarcinoma line. J Appl Toxicol 2000; 20: 21-24.
32. Hong Y, Won J, Lee Y, et al. Melatonin treatment induces interplay of apoptosis, autophagy, and senescence in human colorectal cancer cells. J Pineal Res 2014;56:264–274.
33. Wei J-Y, Li W-M, Zhou L-L, et al. Melatonin induces apoptosis of colorectal cancer cells through HDAC4 nuclear import mediated by CaMKII inactivation. J Pineal Res. 2015;58:429–438.
34. Lee JH, Yoon YM, Han YS, et al. Melatonin promotes apoptosis of oxaliplatin-resistant colorectal cancer cells through inhibition of cellular prion protein. Anticancer Res 2018;38:1993–2000.
35. Zhou N, Wei ZX, Qi ZX.. Inhibition of autophagy triggers melatonin-induced apoptosis in glioblastoma cells. BMC neuroscience, 2019;20(1), 63.
36. Moreno-SanJuan S, Puentes-Pardo JD, Casado J, et al. Agomelatine, a Melatonin-Derived Drug, as a New Strategy for the Treatment of Colorectal Cancer. Antioxidants 2023; 12(4), 926.