Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 78, No 10, 2025

MEDICINE

Oncology

EFFECT OF MELATONIN AND CISPLATIN ON CERVICAL CANCER CELLS: ROLE OF TRPV1 CHANNELS

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Received on March 7, 2025
Presented by D. Damianov, Member of BAS, on July 29, 2025

Abstract

Cisplatin, a widely used treatment for cervical cancer, and melatonin, known for enhancing the apoptotic effects of cisplatin, both play crucial roles in our study. We investigated the combined effects of these two agents on HeLa cervical cancer cells, with focus on the role of TRPV1 channels, which we found to be of significant importance. According to the treatment performed, the cell samples were divided into seven main groups: control, cisplatin, cisplatin + capsazepine (TRPV1 antagonist), melatonin, melatonin + capsazepine, cisplatin + melatonin, and cisplatin + melatonin + capsazepine. Each group was stimulated with capsaicin (TRPV1 agonist) in all analyses. We measured apoptosis, cytosolic calcium, intracellular reactive oxygen species, mitochondrial depolarization, caspase-9, and caspase-3 levels. The combined application of cisplatin and melatonin led to a significant increase in apoptosis levels, which were statistically significantly higher compared to both the control group and the group treated with cisplatin alone ($p < 0.001$) (Fig. 2). This notable increase underscores the potential of this combined treatment in treating cervical cancer. Our findings strongly suggest that melatonin could be a promising adjunct therapy for cisplatin-induced cervical cancer. By elevating cytosolic Ca^{2+} levels, promoting apoptosis, and increasing intracellular ROS levels through TRPV1 channels, melatonin indicated potential as an effective therapeutic option (Fig. 1–4).

Key words: cervix cancer, TRPV1 channel, cisplatin, melatonin, oxidative stress

https://doi.org/10.7546/CRABS.2025.10.10

Introduction. Cervical cancer (CC) is among the most commonly diagnosed cancers in women worldwide, following breast and ovarian cancer, and is the third most frequently occurring cancer in women [1]. The anticancer effect of cisplatin is mediated by interfering with DNA repair mechanisms, resulting in DNA damage that subsequently triggers programmed cell death, or apoptosis, in cancer cells. This process not only facilitates tumour shrinkage but also helps prevent its metastasis within the body [2]. The heightened oxidative stress activates TRP channels, which are located in the cell membrane and are stimulated by oxidative stress, facilitating the influx of cations such as Na^+ and Ca^{2+} into the cell through these channels [3]. Among the TRP channels, which are classified into six subfamilies in mammals – TRPA (Ankyrin), TRPC (Canonical), TRPM (Melastatin), TRPML (Mucolipin), TRPP (Polycystin), and TRPV (Vanilloid) – TRPV channels are cation-permeable and comprise a total of six subtypes (TRPV1–6). The TRPV1 channels can be stimulated by oxidative stress, temperatures of 43 °C or higher, low pH, and capsaicin, the active compound in chili peppers [3, 4].

Melatonin, in addition to being synthesized in various tissues of the body, is primarily produced by pinealocytes in the pineal gland and is known for its

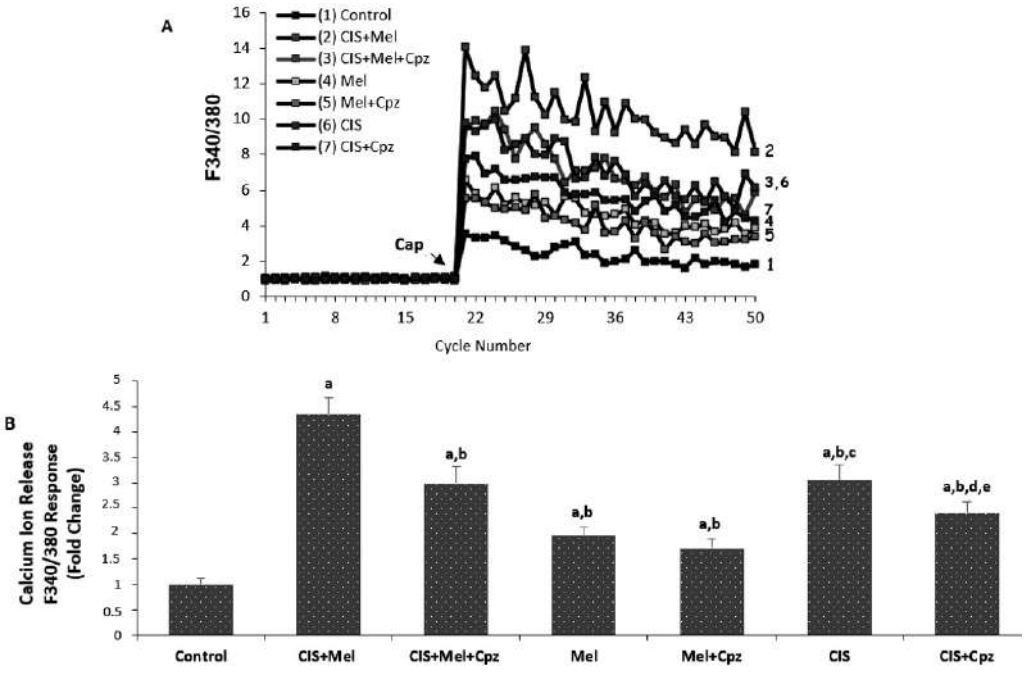

Fig. 1. The effect of cisplatin (CIS, 10 µM, 24 h) and melatonin (Mel, 0.2 mM, 24 h) on cytosolic calcium levels in the HeLa cells. Cells are stimulated by capsaicin (Cap 0.1 mM and on the 20th cycle) but they were inhibited by capsazepine (Cpz 0.1 mM for 30 min) (mean ± SD and $n = 3$). $^{a}p < 0.001$ vs. Control, $^{b}p < 0.001$ vs. CIS + Mel, $^{c}p < 0.001$ and $^{d}p < 0.05$ vs. Mel, $^{e}p < 0.05$ vs. CIS group

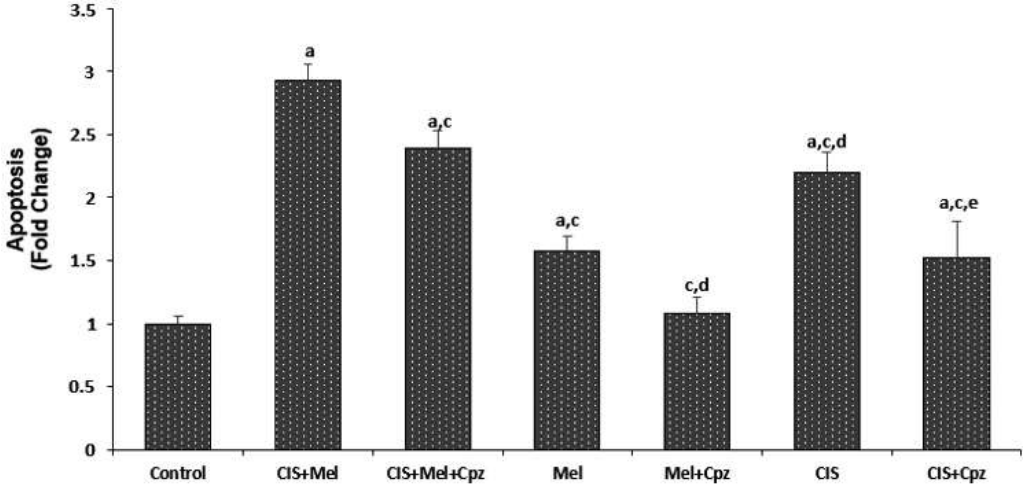

Fig. 2. The effect of cisplatin (CIS, 10 µM, 24 h) and melatonin (Mel, 0.2 mM, 24 h) on apoptosis levels in the HeLa cells. Cells are stimulated by capsaicin (Cap 0.1 mM for 10 min) but they were inhibited by capsazepine (Cpz 0.1 mM for 30 min) (mean ± SD and $n = 10$). $^{a}p < 0.001$ and $^{b}p < 0.05$ vs. Control, $^{c}p < 0.001$ vs. CIS + Mel, $^{d}p < 0.001$ vs. Mel and $^{e}p < 0.001$ vs. CIS

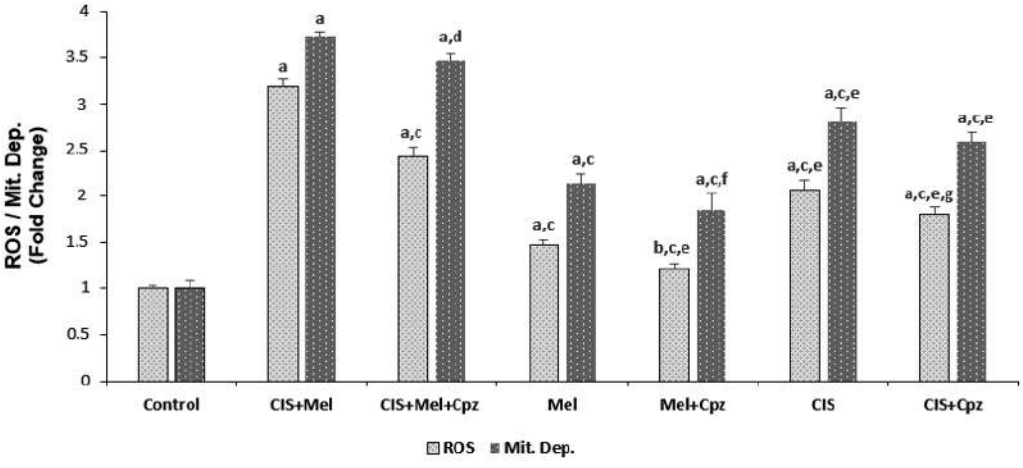

Fig. 3. The effect of cisplatin (CIS, 10 µM, 24 h) and melatonin (Mel, 0.2 mM, 24 h) on reactive oxygen species and mitochondrial depolarization levels in the HeLa cells. Cells are stimulated by capsaicin (Cap 0.1 mM for 10 min) but they were inhibited by capsazepine (Cpz 0.1 mM for 30 min) (mean±SD and $n = 10$). $^{a}p < 0.001$ and $^{b}p < 0.05$ vs. Control, $^{c}p < 0.001$ and $^{d}p < 0.05$ vs. CIS + Mel, $^{e}p < 0.001$ and $^{f}p < 0.05$ vs. Mel and $^{g}p < 0.001$ vs. CIS

antioxidant properties. It has been demonstrated that melatonin not only supports and strengthens the immune system in individuals who experience regular nighttime sleep but also exhibits both protective and anti-cancer effects [5].

In this study, we aimed to investigate the effect of melatonin, which is known for its proapoptotic effects in cancerous cells, in combination with cisplatin, on TRPV1 channels in cervical cancer cells.

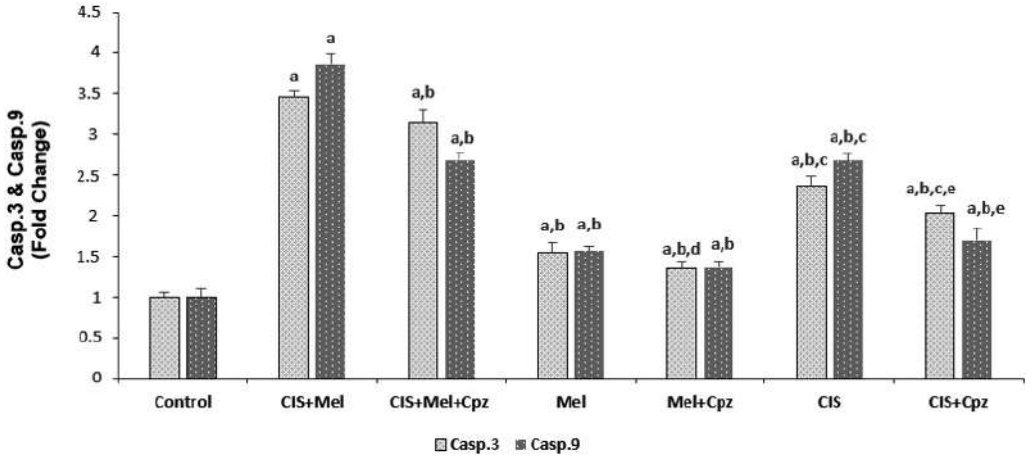

Fig. 4. The effect of cisplatin (CIS, 10 µM, 24 h) and melatonin (Mel, 0.2 mM, 24 h) on caspase-3 and caspase-9 activation levels in the HeLa cells. Cells are stimulated by capsaicin (Cap 0.1 mM for 10 min) but they were inhibited by capsazepine (Cpz 0.1 mM for 30 min) (mean ± SD and $n = 10$). $^{a}p < 0.001$ vs. Control, $^{b}p < 0.001$ vs. CIS + Mel, $^{c}p < 0.001$ and $^{d}p < 0.05$ vs. Mel and $^{d}p < 0.001$ vs. CIS

Materials and methods. ***Reagents/Stains.*** Dulbecco's modified Eagle's medium (DMEM) and Dihydrorhodamine-123 (DHR 123) were procured from Sigma Aldrich (St. Louis, MO). Fura-2 (AM) fluorescent calcium stain was procured from Calbiochem (Darmstadt, Germany), Pluronic® F-127 was procured from Biovision (San Francisco, USA). APO percentage assay with a release buffer was procured from Biocolor (Belfast, Northern Ireland). Probenecid and JC-1 were procured from Santa Cruz (Dallas, Texas, USA). Caspase-3 (AC-DEVD-AMC) and caspase-9 (AC-LEHD-AMC) substrates were procured from Enzo (Lausanne, Switzerland).

Cell culture. HeLa cells (a human cervical epithelioid carcinoma cell line) were obtained from the Şap Institute of the Ministry of Agriculture and Forestry of the Republic of Türkiye (Ankara, Türkiye). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Fisher Scientific) and 1% penicillin–streptomycin antibiotic (Merck) solution. Cultures were maintained in sterile 25 cm^2 flasks with filter caps, each containing 5 mL of medium, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

Once the cells reached 75–85% confluence, they were treated with the chemical agents as described in the study group section. Cultures were monitored daily for signs of contamination. Following treatment, the cells were washed with PBS solution and detached using 0.25% Trypsin-EDTA. Fresh medium (4 mL per flask) was then added. The cell suspensions were collected using an automatic pipette and transferred to 15 mL sterile Falcon tubes. Cells were centrifuged at 100× g

for 5 min. The supernatants were discarded, and the cells were resuspended in fresh medium. A second centrifugation step was performed to wash the cells and prepare them for subsequent experimental procedures.

Dose preferences. In cervical adenocarcinoma cell lines such as HeLa cells, the IC_{50} (half maximal inhibition concentration) for cisplatin is usually reported between 5–20 µM. A dose of 10 µM cisplatin usually provides 'low to moderate' toxicity, sufficient to prevent complete cell death while producing measurable apoptosis and DNA damage. In this way, combination effects (e.g. with melatonin) can be clearly observed. In addition, the clinical translatability of in vitro results is higher, as they fall within a low and clinically relevant dose range.

Melatonin may have both antioxidant and pro-apoptotic effects. This depends on cell type and concentration.

In the range of 0.1–1 mM melatonin can generally increase apoptotic signalling by producing a pro-oxidant effect in cancer cells. In particular, a low dose of 0.2 mM has been favoured in many studies as a dose that enhances the effect of cisplatin or modulates its side effects.

Study groups. HeLa cells were cultured at 37 °C. According to the treatment performed, the cell samples were divided into seven main groups.

Group 1 (Control): The cells were not incubated with cisplatin, melatonin and capsazepine (TRPV1 channels antagonist, Cpz) but were kept in a flask containing the same cell culture medium and conditions.

Group 2 (CIS+Mel): Cells were incubated with 10 µM cisplatin and 0.2 mM melatonin for 24 h.

Group 3 (CIS+Mel+Cpz): Cells were incubated with 10 µM cisplatin and 0.2 mM melatonin for 24 h and then incubated with 0.1 mM capsazepine for 30 min.

Group 4 (Mel): Cells were incubated with 0.2 mM melatonin for 24 h [6].

Group 5 (Mel+Cpz): Cells were incubated with 0.2 mM melatonin for 24 h and then incubated with 0.1 mM capsazepine for 30 min.

Group 6 (CIS): Cells were incubated with 10 µM cisplatin for 24 h [7].

Group 7 (CIS+Cpz): Cells were incubated with 10 µM cisplatin for 24 h and then incubated with 0.1 mM capsazepine for 30 min.

During calcium signalling analysis (Fura-2/AM), cells were stimulated with 20 cycles of 0.1 mM Cap in the presence of 1.2 mM calcium and a calcium-free buffer in the extracellular environment. For apoptosis experiments involving intracellular reactive oxygen species, mitochondrial depolarization, and caspase-3 and caspase-9, the cells were further treated with the TRPV1 channel agonist capsaicin (Cap, 0.1 mM, 10 min) to activate the TRPV1 channel before analysis.

Measurement of intracellular free calcium concentration **($[Ca^{2+}]_i$).** The calcium ion concentration was measured using UV light-excitable Fura-2 acetoxymethyl ester (Fura-2-AM) as an intracellular calcium ion indicator. Fluorescence emission intensity at 510 nm was determined in individual wells using a plate reader equipped with an automated injection system (Synergy™ H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 s for 50 acquisition cycles (cycle: 3 s; gain: 120) in response to agonists (Cap, 0.1 mM) added with the automated injector. $[Ca^{2+}]_i$ in cells was expressed as the average emission at 510 nm in individual wells in response to excitation at 340/380 nm, normalized to initial fluorescence emission obtained during the first 10–20 cycles [8].

Apoptosis and Intracellular ROS production measurement. The APOPercentage™ which is used as an assay for the detection and quantification of apoptosis (Biocolor Ltd., Belfast, Northern Ireland) was performed according to the manufacturer instruction. The APOPercentage™ assay is a dye-uptake assay, which stains only the apoptotic cells with a red dye. When the membrane of apoptotic cell lost its asymmetry, the APOPercentage dye is bonded phosphatidyl serine lipids actively and transported into cells, staining apoptotic cells red, thus allowing the detection of apoptosis by a multiplate reader (Synergy™ H1, Biotek, USA) as previously described elsewhere [8].

In reactive oxygen radicals production analysis, Rhodamine 123 (Rh 123) is a non-fluorescent, non-charged dye that can easily pass the cell membranes, where it is oxidized to cationic rhodamine 123, which localizes in the mitochondria and exhibits green fluorescence. The excitation and emission wavelengths for the analysis were 488 nm and 543 nm, respectively. Data are presented as fold-change over the pretreatment level [8].

Assay for caspase-3 and caspase-9 activities. The determinations of caspase-3 and caspase-9 activities were based on methods previously reported [9]. Caspase-3 substrate (N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-Methylcoumarin) (AC-DEVD-AMC) and caspase-9 substrate (Ac-Leu-Glu-His-Asp-7-Amino-4-methylcoumarin) (AC-LEHD-AMC) cleavages were measured with the microplate reader (Synergy™ H1, Biotek, USA) with excitation wavelength of 360 nm and emission at 460 nm.

Mitochondrial membrane potential (JC-1) analyses. The lipophilic cationic dye, JC-1, exhibits potential-dependent accumulation in mitochondria. It indicates mitochondrial depolarization by a decrease in the ratio of red-to-green fluorescence intensity. The green JC-1 signal was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, and the red signal was measured at an excitation wavelength of 540 nm and an emission wavelength of 590 nm [10].

Statistical analyses. All results were expressed as means ± standard deviation (SD). Significant differences between the groups were assessed using one-way ANOVA. Statistical analyses were calculated using GraphPad Prism version 7.04

for Windows (GraphPad Software, San Diego, California, USA) and $p < 0.05$ was considered significant.

Results. Ca^{2+} concentration, programmed cell death values, ROS and mitochondrial depolarization levels, and caspase-3 and caspase-9 levels in HeLa cells were significantly higher in the CIS + Mel, Mel, and CIS groups compared to the control group ($p < 0.001$). The Ca^{2+} level, programmed cell death values, ROS and mitochondrial depolarization levels, and caspase-3 and caspase-9 levels in the CIS + Mel group were significantly higher than in the Mel and CIS groups ($p < 0.001$). When comparing the Mel and CIS groups, the Ca^{2+} level, programmed cell death values, ROS and mitochondrial depolarization levels, and caspase-3 and caspase-9 levels were lower in the Mel group than in the CIS group ($p < 0.001$). Furthermore, when the CIS + Mel group was compared to the CIS + Mel + Cpz group (treated with the TRPV1 channel inhibitor capsazepine), and the CIS group was compared to the CIS + Cpz group, the groups treated with capsazepine exhibited significantly lower cytosolic calcium levels, programmed cell death values, ROS and mitochondrial depolarization levels and caspase-3 and caspase-9 levels than those without capsazepine ($p < 0.001$; $p < 0.05$) (Fig. 1–4). For calcium signalling, however, when the Mel group was compared to the Mel+Cpz group, no statistically significant difference was observed between them. In other analyses, Mel groups had higher values than Mel+Cpz groups ($p < 0.001$) (Fig. 1).

Discussion. Evidence from in vitro studies supports that TRPV1 channels influence proliferation, invasion, and migration in CC cells. TRPM4 channels regulate cell proliferation and invasion, while TRPM7 channels are involved in apoptosis and cell growth. Additionally, TRPM8 channels affect tumour cell adhesion [9]. Based on the evidence from various studies, the hypothesis that TRP channels should be primary targets in cancer treatment has gained increasing support. Research on activating or inhibiting TRP channels in various cancer cell types has revealed that these channels play essential roles in cancer therapy, including chemotherapy, immunotherapy, and radiotherapy [10]. In this study, we exhibited that increased TRPV1 channel activation in CC cells has a direct impact on mitochondrial depolarization and intracellular ROS levels through calcium signalling, which plays a critical role in tumour cell pathophysiology and apoptosis (Fig. 1, 3).

TRP channels, which have been implicated in key tumour cell functions such as proliferation and invasion, are affected during chemotherapy in cancer cells [10]. In a study conducted on mice, elevated levels of TRPA1 and TRPV1 channels were detected in tumour cells during apoptosis induced by cisplatin treatment, with TRPM8 upregulation occurring at a later stage (48–72 h). Additionally, TRP channels have been identified as key mediators in the transcriptional regulation of dorsal root ganglia, which play a significant pathophysiological role in pain and peripheral neuropathies, major side effects of cisplatin [11]. A fur-

ther study on cisplatin-induced ototoxicity indicated that cisplatin increases ROS levels in cochlear cells, leading to ototoxicity, and that TRPV1 channels act as crucial mediators in this process [4]. In another study, TRPA1 activation and overexpression led to a dose-dependent increase in intracellular calcium levels, subsequently enhancing apoptosis in renal cells [12]. According to the findings of this research, we observed that during cisplatin administration in CC cells, TRP channel activation significantly elevated intracellular ROS levels, cytosolic calcium concentrations, mitochondrial depolarization, and the activation of caspase-3 and caspase-9 compared to cisplatin alone (Fig. 1–4). These findings highlight the potential significance of targeting TRPV1 channels to enhance the efficacy of cisplatin in the treatment of cervical cancer.

Melatonin has been shown in in vitro studies to have direct and positive effects on mitochondrial dysfunction, oxidative stress, and programmed apoptosis [7]. In the development of cervical cancers, where HPV infections are an important etiological factor, the mechanisms underlying HPV-related cancer development include oxidative stress, increased intracellular ROS, DNA damage, increased mutations, and activation of growth factors. Melatonin's protective effects in cervical cancers are primarily related to its antioxidant properties, affecting intracellular ROS accumulation and apoptosis [13]. Preclinical and clinical studies have outlined the positive effects of melatonin use during chemotherapy with various cancer types and agents on treatment outcomes.

It has been reported that melatonin exhibits a synergistic effect with cisplatin in the treatment of ovarian, breast, and bone cancers, enhancing the drug's efficacy [14, 15]. Although limited in number, studies in the literature have examined the role of TRP channels in the anticancer effects of melatonin and its use in combination with chemotherapeutic agents. In an animal study, the role of TRP channels in reducing orofacial nociception was investigated. Although interactions between melatonin and TRPA1 and TRPM8 channels were observed, the strongest interaction was found with the TRPV1 channels [16]. A separate study reported that melatonin reduced oxidative neurotoxicity and neuropathic pain during docetaxel treatment in mice, and the activation of the TRPA1 channel enhanced these effects of melatonin [17]. In yet another investigation on MCF-7 breast cancer cells, it was shown that melatonin increased the oxidative stress and apoptotic effects of doxorubicin in tumour cells, and the activation of the TRPV1 channel had a synergistic effect, further enhancing this activity [18]. Our present research highlights that melatonin, both alone and in combination with cisplatin, induced apoptosis in tumour cells, and the activation of TRPV1 channels further enhanced these effects (Fig. 2).

In conclusion, our study investigating the antitumour effects of melatonin on cervical cancer cells, its possible effects with chemotherapeutic agents and the roles of TRPV1 channels in these processes revealed that melatonin showed a direct antitumour effect on CC cells. Furthermore, TRPV1 channels were shown to

positively mediate the antitumour effects in cervical cancer cells in both melatonin, cisplatin and combination therapies and it was concluded that TRPV1 channels could be targeted during chemotherapeutic drug use in these cancer cells.

REFERENCES

[1] Zhang S., H. Xu, L. Zhang, Y. Qiao (2020) Cervical cancer: Epidemiology, risk factors and screening, Chin. J. Cancer Res., **32**(6), 720–728.

[2] Nazıroğlu M., N. Braidy (2017) Thermo-sensitive TRP channels: Novel targets for treating chemotherapy-induced peripheral pain, Front. Physiol., **8**, 1040.

[3] Marini M., M. Titiz, D. S. Monteiro de Araújo et al. (2023) TRP channels in cancer: Signaling mechanisms and translational approaches, Biomolecules, **13**(10), 1557.

[4] Mukherjea D., S. Jajoo, C. Whitworth et al. (2008) Short interfering RNA against transient receptor potential vanilloid 1 attenuates cisplatin-induced hearing loss in the rat, J. Neurosci., **28**(49), 13056–13065.

[5] Wang L., C. Wang, W. S. Choi (2022) Use of melatonin in cancer treatment: Where are we?, Int. J. Mol. Sci., **23**(7), 3779.

[6] Yun C. W., S. Kim, J. H. Lee, S. H. Lee (2018) Melatonin promotes apoptosis of colorectal cancer cells via superoxide-mediated ER stress by inhibiting cellular prion protein expression, Anticancer Res., **38**(7), 3951–3960.

[7] Bostan M., M. Mihaila, G. G. Petrica-Matei et al. (2021) Resveratrol modulation of apoptosis and cell cycle response to cisplatin in head and neck cancer cell lines, Int. J. Mol. Sci., **22**(12), 6322.

[8] Öz A., Ö. Çelik (2016) Curcumin inhibits oxidative stress-induced TRPM2 channel activation, calcium ion entry and apoptosis values in SH-SY5Y neuroblastoma cells: Involvement of transfection procedure, Mol. Membr. Biol., **33**(3–5), 76–88.

[9] Jiang S., X. Lin, Q. Wu et al. (2024) Transient receptor potential channels' genes forecast cervical cancer outcomes and illuminate its impact on tumor cells, Front. Genet., **15**, 1391842.

[10] Neuberger A., M. Oda, Y. A. Nikolaev et al. (2023) Human TRPV1 structure and inhibition by the analgesic SB-366791, Nat. Commun., **14**(1), 2451.

[11] Ta L. E., A. J. Bieber, S. M. Carlton et al. (2010) Transient receptor potential vanilloid 1 is essential for cisplatin-induced heat hyperalgesia in mice, Mol. Pain, **6**, 15.

[12] Meng X., Y. Zhang, Y. Ma et al. (2021) Transient receptor potential ankyrin 1 mediates cisplatin-induced apoptosis in renal tubular cells via calcium-dependent signaling pathway, Ann. Palliat. Med., **10**(8), 9025–9038.

[13] Shafabakhsh R., R. J. Reiter, H. Mirzaei et al. (2019) Melatonin: A new inhibitor agent for cervical cancer treatment, J. Cell Physiol., **234**(12), 21670–21682.

[14] Zemła A., I. Grzegorek, P. Dzięgiel, K. Jabłońska (2017) Melatonin synergizes the chemotherapeutic effect of cisplatin in ovarian cancer cells independently of MT1 melatonin receptors, In Vivo, **31**(5), 801–809.

[15] Hosseini F., D. Shanehbandi, J. Soleimanpour et al. (2022) Melatonin increases the sensitivity of osteosarcoma cells to chemotherapy drug cisplatin, Drug Res. (Stuttg), **72**(6), 312–318.

[16] Silva Sampaio G. M. M., A. E. Vieira-Neto, G. O. Leite, A. R. Campos (2023) Melatonin promotes orofacial antinociception in adult zebrafish by modulating TRP channels, Physiol. Behav., **269**, 114238.
[17] Ertilav K., M. Naziroğlu, Z. S. Ataizi, K. Yildizhan (2021) Melatonin and selenium suppress docetaxel-induced TRPV1 activation, neuropathic pain and oxidative neurotoxicity in mice, Biol. Trace Elem. Res., **199**(4), 1469–1487.
[18] Koşar P. A., M. Naziroğlu, İ. S. Övey, B. Çiğ (2016) Synergic effects of doxorubicin and melatonin on apoptosis and mitochondrial oxidative stress in MCF-7 breast cancer cells: Involvement of TRPV1 channels, J. Membr. Biol., **249**(1–2), 129–140.

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