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The effect of melatonin on the barrier properties of human primary aortic endothelial and human aortic smooth muscle cells based on the real-time cell electric impedance sensing system

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ABSTRACT

Introduction: Experimental studies have confirmed the potential vasculoprotective effects of melatonin. The aim of the study was to evaluate the influence of melatonin and its modulative role on 7-ketocholesterol-induced changes in human aortic endothelial cells (HAECs) and human aortic smooth muscle cells (HuAoSMCs).

Materials and methods: The real-time cell electric impedance sensing (xCELLigence) system was used to measure cell impedance, while flow cytometry was employed to assess viability and apoptosis.

Results: It was found that melatonin did not change the impedance and viability of HuAoSMCs, nor did it inhibit their 7-ketocholesterol-induced apoptosis. Melatonin decreased the impedance of HAECs. However, this effect was not attributed to cell apoptosis. In addition, it did not prevent the 7-ketocholesterol--induced decrease in HAEC impedance. Nevertheless, pretreatment with melatonin at concentrations of 10 nM and 100 nM attenuated the apoptosis caused by 7-ketocholesterol.

Conclusions: This study confirmed the toxic effect of 7-ketocholesterol on primary human vascular wall cells, using 2 independent methods. We have demonstrated that melatonin has no barrier-protective effect, but low concentrations of melatonin may counteract the toxic effect of 7-ketocholesterol on HAECs through the inhibition of apoptosis. Melatonin has no significant effect on the impedance and viability of HuAoSMCs.

Keywords: melatonin; 7-ketocholesterol; HAEC; HuAoSMc; apoptosis; impedance.

INTRODUCTION

Melatonin, a neurohormone synthesized by the pineal gland, exhibits chronobiotic properties. In addition to its well-known role in regulating circadian rhythms, it plays a wide range of roles in many vital physiological and pathological processes. Numerous experimental studies have demonstrated that melatonin has oncostatic, immunomodulatory, antiproliferative [1, 2], antioxidant [3, 4], and anti-inflammatory effects [5, 6], and may play an important role in angiogenesis [7]. Melatonin has been shown to protect the aorta against nicotine-induced damage, inhibit neointima formation, maintain homeostasis, and attenuate alcohol-induced vasculopathy [8]. Moreover, it has been found to lower blood pressure [9, 10] by interacting with a distinct family of G-protein coupled receptors, including MT1 and MT2 which are present on vascular wall cells [11, 12]. The MT1 melatonin receptor interacts with Gi and Gq/11 G proteins, which are sensitive and insensitive to pertussis toxin, respectively. It hinders cyclic adenosine monophosphate (cAMP) and protein kinase A signaling, as well as the phosphorylation of cAMP response element-binding protein, when stimulated by forskolin. Additionally, the MT1 receptor promotes the phosphorylation of mitogen-activated protein kinase 1/2 and extracellular signal-regulated kinase 1/2, and enhances potassium conductance through Kir inwardly rectifying channels. Activation of the MT2 melatonin receptor suppresses both cAMP production and guasine 3' 5'-cyclic monophosphate formation upon stimulation by forskolin. It induces the activation of protein kinase C in the suprachiasmatic nucleus and reduces the calcium-dependent dopamine release in the retina [13]. Furthermore, melatonin exhibits barrier-protective properties [14, 15] which may play a crucial role in the pathogenesis of atherosclerosis.

Endothelial damage is recognized as the initial step in the development of vascular lesions. Seven-ketocholesterol, a prominent product of sterol oxidation, is commonly found in atherosclerotic plaques. This compound demonstrates high cytotoxicity and induces apoptosis of both endothelial cells (ECs) and smooth vascular cells [16, 17]. It can trigger the release of cytochrome c and induce caspase activation in ECs [18]. Moreover, it inhibits the proliferation of ECs [19] and compromises the barrier properties of the endothelial monolayer, leading to an increased number of non-adhering cells [16, 17]. Considering the diverse effects of melatonin, including its vasoprotective

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properties, it is hypothesized that melatonin may counteract the toxic effects of 7-ketocholesterol on endothelial and smooth vascular cells. However, no studies have investigated whether melatonin can protect human aortic smooth muscle cells (HuAoSMCs) against the toxic effects of 7-ketocholesterol.

Therefore, the aim of this study was to assess the impact of melatonin on changes induced by 7-ketocholesterol in human aortic endothelial cells (HAECs) and HuAoSMCs. To achieve this, cell impedance was measured using the real-time cell electric impedance sensing (xCELLigence) system, while viability and apoptosis were evaluated through flow cytometry.

MATERIALS AND METHODS

All the experimental procedures described in the study were performed in accordance with the Declaration of Helsinki and approved by Bioethical Committee of the Medical University in Łódź Consent number RNN/174/12/KB.

Cell culture

The primary HAECs and primary HuAoSMCs were obtained from Lonza and were cultured until reaching full confluence at 37°C in a humidified atmosphere of 95% air – 5% CO2 for all experiments. The HAECs were cultured in EC basal medium-2 containing the following growth supplements: hydrocortisone, human fibroblast growth factor basic (hFGF-B), vascular endothelial growth factor (VEGF), Long Arg3 insulin-like growth factor-I (R3-IGF-1), ascorbic acid, heparin, fetal bovine serum (FBS), human epidermal growth factor (hEGF), and Gentamicin sulfate-Amphotericin (GA-1000) (Lonza). The HuAoSMCs were grown in smooth muscle cell basal medium-2 supplemented with hEGF, insulin, hFGF-B, FBS, and GA-1000 (Lonza). All cells were cultured following the manufacturer's instructions (Lonza).

After 3 passages, the cells were detached from the flasks using a 0.025% trypsin – ethylenediaminetetraacetic acid solution (Sigma-Aldrich). They were then seeded in 12-well tissue culture plates (Nunc) for flow cytometry analysis or in E plate-16 for real-time monitoring using the xCELLigence system (Roche).

Cell treatments

Seven-ketocholesterol and melatonin were purchased from Sigma-Aldrich. They were dissolved in ethanol and added to the medium to obtain a final concentration of 40 μg/mL of 7-ketocholesterol and 10 nM, 100 nM and 100 μM of melatonin. Previous scientific reports have demonstrated that the solvent concentrations used in our study are non-toxic [16, 17].

Real-time cell electric impedance sensing

The xCELLigence system (Roche Applied Science) is based on tracking electrical impedance signals, which enables the real-time monitoring of cell growth on microelectrode-coated plates. The impedance readout is expressed in arbitrary units as the cell index (CI), reflecting changes in barrier properties, monolayer permeability, cell number, viability, adhesion, and morphology. The CI calculation is based on the following formula:

$$CI = (Zi - Zo)/15,$$

where: *Zi* – the impedance at an individual time point during the experiment, *Zo* – represents the impedance at the start of the experiment

The normalized cell index (nCI) at a certain time point is acquired by dividing the CI value by the value at a reference time point.

Human aortic endothelial cells were separately seeded on E-16 plates at a density of 10,000 cells per well, while the HuAoSMCs were seeded at a density of 15,000. The cells were monitored every hour after seeding till they reached the plateau phase.

After 27 h, the HAECs reached a stable plateau. At that point, 100 μL of culture medium was removed from each well and carefully replaced with 100 μL of medium containing melatonin at concentrations of 20 nM, 200 nM, and 200 μM, resulting in a final melatonin concentration of 10 nM, 100 nM, or 100 μM per well. The xCELLigence system was used to monitor the influence of the different melatonin concentrations on the CI for 48 h, and to analyze the effect of melatonin on the changes in HAEC impedance 5 and 48 h after stimulation. The nCI was estimated using the last point before stimulation with melatonin.

To evaluate the modulatory role of melatonin on damage induced by 7-ketocholesterol, the experiment was paused after 14 h, and 100 μL of medium containing melatonin was removed. In exchange, 100 μL of culture medium was added with 80 μg/mL 7-ketocholesterol and either 10 nM, 100 nM, or 100 μM of melatonin. The nCI was calculated using the last measurement of CI before the addition of 7-ketocholesterol.

Human aortic smooth muscle cells reached a stable plateau phase after 21 h. To assess the effect of melatonin on the impedance of HuAoSMCs, 100 μL of culture medium was replaced with 100 μL of medium containing melatonin at a concentration of 20 nM, 200 nM, or 200 μM. Impedance was measured every 30 min during the next 32 h. The nCI was analyzed using the last point before melatonin treatment.

In addition, the modulatory influence of melatonin on HuAoSMCs treated with 7-ketocholesterol was also examined. Cells were pretreated for 6 h with melatonin at concentrations of 10 nM, 100 nM, or 100 μM and then stimulated with 7-ketocholesterol (40 μg/mL) with an appropriate melatonin concentration. Impedance was measured for the next 26 h. The nCI was analyzed using the last measurement before the addition of 7-ketocholesterol.

Flow cytometry analysis of apoptosis

Human aortic endothelial cells and HuAoSMCs were seeded in 12-well culture plates (Nunc) and incubated for 5–7 days until they reached full confluence. At this stage, the cells were treated with 7-ketocholesterol (40 μg/mL) or with melatonin at a concentration of 10 nM or 100 μM for 48 h. In separate experiments, the cells were pretreated with increasing

concentrations of melatonin (10 nM, 100 nM, 100 µM) for 4 h and subsequently stimulated with 7-ketocholesterol at a concentration of 40 µg/mL.

After 48 h of stimulation with melatonin, both HAECs and HuAoSMCs were trypsinized. Apoptosis was then detected using Annexin V (Ax5-FITC) and propidium iodide – PI (FITC annexin V Apoptosis detection kit, BD Pharmingen). The cell suspension was examined using a Beckman-Coulter FC500 flow cytometer (Władysław Biegański Hospital Laboratory, Łódź, Poland). Ax5-FITC(–) and PI(–) cells were considered living cells, Ax5-FITC(+) and PI(–) cells were considered early apoptotic cells, Ax5-FITC(+) and PI(+) cells were considered late-apoptotic cells, and Ax5-FITC(–) and PI(+) cells were considered as necrotic cells. The cells were divided into PI(+) and Ax5-FITC(+) groups for statistical analysis.

Statistical analysis

All experiments were performed at least 6 times. The results are expressed as mean ±standard error of measurement (SEM). Statistical analysis was conducted using GraphPad Prism 5 software. One-way ANOVA followed by Tukey's *post hoc* analysis was used to analyze the results. Statistical significance was determined at $p < 0.05$.

RESULTS

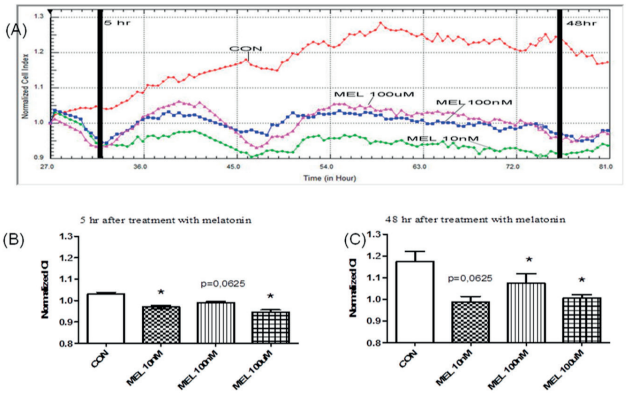
Melatonin destabilizes the integrity of the endothelial monolayer, as analyzed by the xCELLigence system

A xCELLigence system was used to monitor dynamic changes in the barrier properties of HAEC induced by different concentrations of melatonin (10 nM, 100 nM, 100 µM) – Figure 1C. After 5 h of culture, the nCI value in unstimulated HAECs was found to be 1.030 ±0.008700, which was significantly higher ($p < 0.05$) than the nCI values observed in HAECs induced by melatonin at concentrations of 10 nM (0.9701 ±0.007343), 100 nM (0.9897 ±0.005425), and 100 µM (0.9465 ±0.01052). The nCI value did not show a significant change in HAECs induced by 100 nM melatonin (0.9897 ±0.005425) – Figure 1A.

After 48 h of culture, the nCI value of unstimulated cells was 1.174 ±0.04746. In 10 nM melatonin-induced HAECs the values were found to be 0.9868 ±0.02693 ($p < 0.05$) and 1.005 ±0.01686 in 100 µM melatonin-induced HAECs ($p < 0.05$). Similar to the previous observation, the nCI value did not show a significant change in HAECs induced by 100 nM melatonin (1.074 ±0.04402) – Figure 1C.

Melatonin decreases barrier properties of human aortic smooth muscle cells as observed using the xCELLigence system

After 32 h of stimulation with 100 µM melatonin, the nCI values (1.172 ±0.04918) were significantly lower than nCI values of unstimulated HuAoSMCs (1.324 ±0.02763, $p < 0.05$). However, lower concentrations of melatonin did not cause significant changes in HuAoSMC impedance (Fig. 2).



* $p < 0.05$ significant differences between treated groups with melatonin and control groups

FIGURE 1. The changes in normalized cell index in unstimulated human aortic endothelial cells (control group) and human aortic endothelial cells induced by 10 nM, 100 nM, and 100 µM melatonin during 48 h of culture in real-time cell electric impedance sensing system

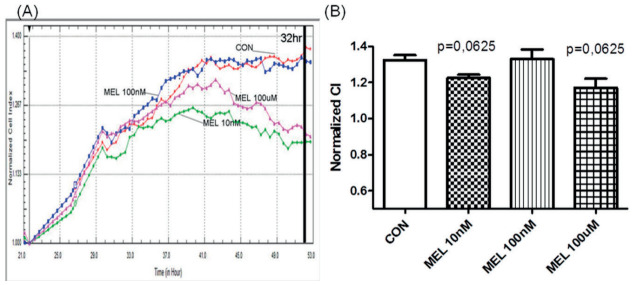


FIGURE 2. The changes in normalized cell index in unstimulated human aortic smooth muscle cells (control group) and human aortic smooth muscle cells induced by 10 nM, 100 nM, and 100 µM melatonin during 32 h of culture in real-time cell electric impedance sensing system

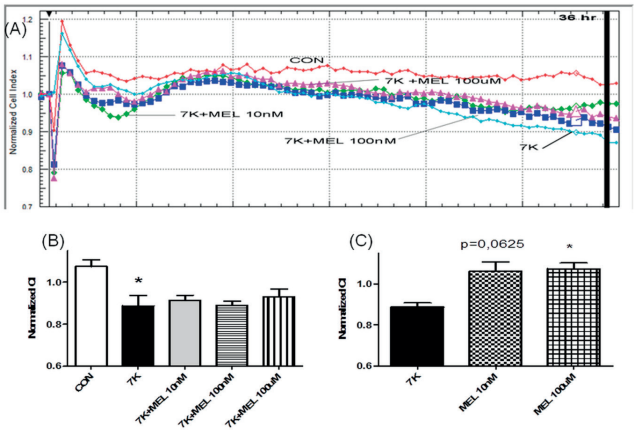
Melatonin did not modify the integrity of 7-ketocholesterol-induced human aortic endothelial cells as observed using the xCELLigence system

A xCELLigence system was used to assess the influence of melatonin on the barrier properties of HAECs induced by 7-ketocholesterol (Fig. 3A). After 36 h of culture, the nCI value in unstimulated HAECs (control group) was 1.074 ±0.03112, while it was 0.8867 ±0.02028 in HAECs induced by 7-ketocholesterol, and this difference was statistically significant ($p < 0.05$) – Figure 3B. However, pretreatment with melatonin, regardless of its concentration, did not significantly modify the nCI value of 7-ketocholesterol-induced HAECs (Fig. 3B).

Melatonin did not modify barrier properties of 7-ketocholesterol-induced human aortic smooth muscle cells, as observed using the xCELLigence system

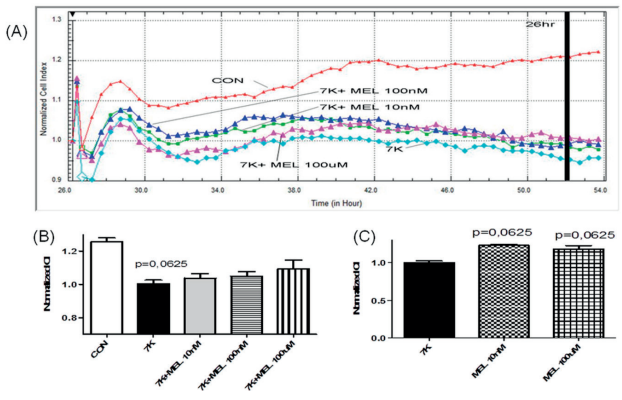
A xCELLigence system was used to assess the influence of melatonin on the barrier properties of HuAoSMCs after the application of 7-ketocholesterol (Fig. 4A). The nCI value in unstimulated HuAoSMCs (control group) was 1.256 ±0.02363, whereas it reached 1.004 ±0.02226 in HuAoSMCs after the application

of 7-ketocholesterol, and this difference was statistically significant ($p < 0.05$) – Figure 4B. However, pretreatment with melatonin, irrespective of its concentration, did not significantly modify the nCI value of 7-ketocholesterol-induced HuAoSMCs (Fig. 4).



* $p < 0.05$ significant differences between 7-ketocholesterol treated groups and control groups (A, B)
Comparison of nCl values between 7-ketocholesterol-induced and melatonin-induced HAECs after 36 h of culture in real-time cell electric impedance sensing system.
* $p < 0.05$ significant differences between the 7-ketocholesterol-treated groups and melatonin-stimulated groups (C)

FIGURE 3. The normalized cell index changes in unstimulated human aortic endothelial cells (control group), human aortic endothelial cells stimulated by 7-ketocholesterol, and human aortic endothelial cells stimulated by melatonin (10 nM, 100 nM, 100 μM) in the presence of 7-ketocholesterol after 36 h of culture using the real-time cell electric impedance sensing system



Comparison of nCl values between 7-ketocholesterol-induced and melatonin-induced HuAoSMCs (C)

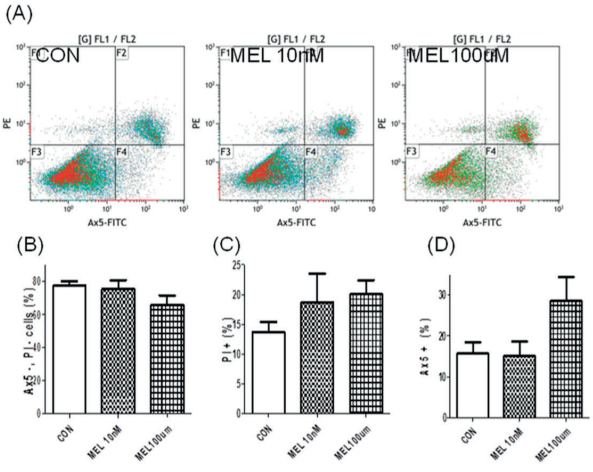
FIGURE 4. The changes in normalized cell index changes in unstimulated (control group) human aortic smooth muscle cells, human aortic smooth muscle cells stimulated by 7-ketocholesterol, and human aortic smooth muscle cells stimulated by melatonin (10 nM, 100 nM, and 100 μM) in the presence of 7-ketocholesterol during 26 h (A, B)

Melatonin did not have an effect on the viability and apoptosis of human aortic endothelial cells, as observed in flow cytometry analysis

The viability and apoptosis of HAECs, either unstimulated or after exposure to melatonin, were assessed after 48 h of culture using flow cytometry. The results demonstrated that stimulation with melatonin at different concentrations (10 nM or 100 μM) did not impact the viability or apoptosis of HAECs. Treatment with 7-ketocholesterol resulted in a decreased in viable cells (80.16 ±3.550 vs. 57.69 ±3.260, $p < 0.05$), an increase in Ax5-FITC(+) cells (13.39 ±3.130 vs. 32.10 ±5.264, $p < 0.05$) and an increase in PI(+) cells (12.42 ±2.169 vs. 25.81 ±4.062, $p < 0.05$) – Figure 5.

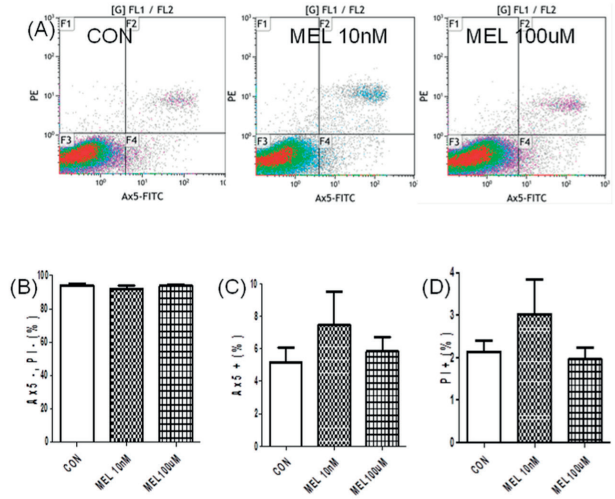
Melatonin did not have an effect on the viability or apoptosis of human aortic smooth muscle cells, as observed in flow cytometry

There were no significant differences in HuAoSMC viability after treatment with melatonin for 48 h in comparison to untreated cells. Exposure to 7-ketocholesterol resulted in a decrease in viable cells (93.95 ±1.079 vs. 80.29 ±4.121, $p < 0.05$), as well as an increase in PI(+) (2.205 ±0.3098 vs. 6.063 ±1.158, $p < 0.05$) and Ax5-FITC(+) cells (5.437 ±1.021 vs. 18.51 ±4.304, $p < 0.05$) – Figure 6.



CON – control group consisting of unstimulated human aortic endothelial cells

FIGURE 5. The effect of melatonin (10 nM, 100 μM) on the viability and apoptosis of human aortic endothelial cells in the flow cytometry analysis

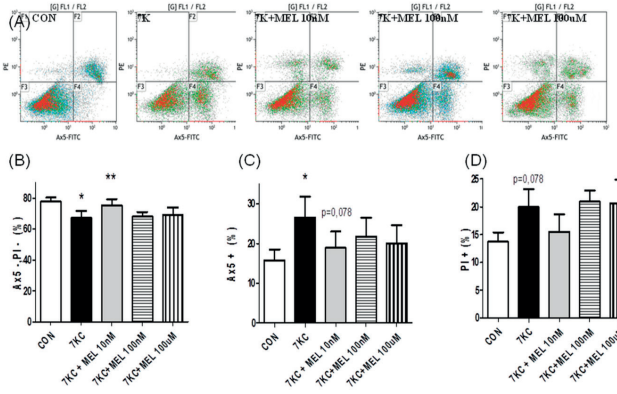


CON – control group consisting of unstimulated human aortic smooth muscle cells

FIGURE 6. The effect of melatonin (10nM, 100μM) on the viability and apoptosis of human aortic smooth muscle cells, as observed in flow cytometry analysis

Melatonin attenuated the apoptosis of 7-ketocholesterol-induced human aortic endothelial cells, as observed in flow cytometry analysis

When HAECs were pretreated with melatonin at a concentration of 10 nM before the addition of 7-ketocholesterol, a significant increase in the number of viable cell number was observed (57.69 ±3.260 vs. 74.27 ±3.649, $p < 0.05$) along with a reduction in the number of PI(+) cells (25.81 ±4.062 vs. 14.68 ±2.850, $p < 0.05$). The higher concentration of melatonin (100 μM) also modified the pronecrotic effects of 7-ketocholesterol on HAEC. Although fewer PI(+) cells were found (25.81 ±4.062 vs. 15.17 ±2.515, $p < 0.05$), the percentage of viable cells was not significantly different (57.69 ±3.260 vs. 73.78 ±5.414, $p > 0.05$) – Figure 7.



* $p < 0.05$ significant differences between groups treated with 7-ketocholesterol and the control groups (unstimulated human aortic endothelial cells)
** $p < 0.05$ significant differences between groups treated with 7-ketocholesterol and the cells pretreated with melatonin (A–D)

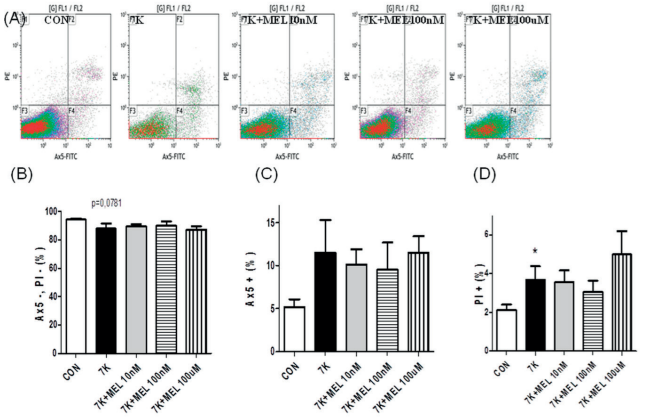
FIGURE 7. The effect of 7-ketocholesterol and 7-ketocholesterol with melatonin on the viability and apoptosis of human aortic endothelial cells, as observed in flow cytometry analysis

Melatonin did not have an effect on the viability and apoptosis of 7-ketocholesterol-induced human aortic smooth muscle cells, as observed in flow cytometry analysis

When melatonin was added before the induction of 7-ketocholesterol, it was not found to have any modulating effect on the response of HuAoSMCs (Fig. 8).

DISCUSSION

This study is the first to utilize the xCELLigence system to evaluate the effect of melatonin on HAECs. The results demonstrate that the endothelial monolayer is susceptible to melatonin, and melatonin-stimulated cells exhibit lower CI values compared to non-stimulated ones. The changes in cellular impedance, as reflected by CI, can be attributed to various phenomena. Decreased cellular impedance may be a result of cellular death [20] or inhibition of proliferation [21]. Reduced CI may be also indicated increased monolayer permeability [22] and cytoskeleton reorganisation [23]. Melatonin has been found to exert a barrier-protective effect on human umbilical vein endothelial cells (HUVECs) following the damaging effect of IL-1β, which influences cytoskeleton reorganisation [14]. In



* $p < 0.05$ significant differences between 7-ketocholesterol-treated groups and the control groups (unstimulated human aortic smooth muscle cells)

FIGURE 8. The effect of 7-ketocholesterol and 7-ketocholesterol with melatonin on the viability and apoptosis of human aortic smooth muscle cells, as observed in flow cytometry analysis

animal models, melatonin has been shown to decrease vessel permeability caused by some factors such as leukotriene B4 [24] or Bacillus Calmette–Guerin [25]. Therefore, melatonin exposure would be expected to increase CI level. However, the observed decrease in CI observed in the study may be due to the inhibition of ECs proliferation or intensification of cellular death, which outweighs the barrier-protective effect of melatonin. Previous studies have demonstrated that melatonin inhibits pro-angiogenic cytokines, including VEGF, epidermal growth factor and insulin-like growth factor [26]. Low concentrations of melatonin (nM) inhibit the cellular cycle of HUVECs and influence kinases responsible for mitogenesis, including those belonging to the of mitogen-activated protein kinases family [27, 28]. Melatonin may also enhance the processes of apoptosis in HUVECs by influencing the expression of p53, Bax, and Bcl-2 proteins [28]. However, contrary to previous reports on the proapoptotic effect of melatonin on ECs, no melatonin-induced apoptosis or necrosis was confirmed in the present study using flow cytometry with PI and Ax5-FITC. Therefore, it is possible that the reduction CI was more likely caused by the inhibition of cellular proliferation.

The xCELLigence analysis revealed that 7-ketocholesterol led to a reduction in CI, indicating cell death Flow cytometry analysis showed that 7-ketocholesterol reduced the viability of HAECs and increased the number of Ax5(+) and PI(+) cells, further suggesting cell death. Previous studies have reported the proapoptotic effect of 7-ketocholesterol on HUVECs [16, 17].

While the xCELLigence system did not confirm the modulating effect of melatonin on 7-ketocholesterol, flow cytometry analysis indicated that 10 nM melatonin had a protective effect, increasing the ratio of viable cells and reducing necrosis. Higher concentrations of melatonin also increased HAEC viability, with a concentration of 100 nM effectively reducing the number of necrotic cells. The observed antinecrotic effect of melatonin observed in this study may be due to its antioxidant properties. Although there are no scientific reports on the impact of

melatonin on processes associated with HAEC death, studies have demonstrated its protective effects against apoptosis in astrocytes by inhibiting mitochondrial dysfunction and reducing reactive oxygen species production [29]. Melatonin has also been shown to protect microvascular ECs against oxidative stress injury and inhibit mitochondrial-dependent cell death [4]. Additionally, melatonin can directly affect apoptosis-associated proteins such as Bax and Bcl-2 [30]. Bax, a proapoptotic protein, induces cell death by creating pores in the mitochondrial membrane, which inhibits the activity of the anti-apoptotic protein Bcl-2. Melatonin added to the medium of a U937 monocytic cells treated with a pro-apoptotic factor has been shown to maintain Bax protein in an inactive monomeric form, blocking the release of mitochondrial cytochrome c and protecting cells from apoptosis [31].

It is important to note that melatonin can exhibit both proapoptotic and anti-apoptotic properties, depending on the concentration and cell type. Reports have shown that at very high concentrations (1 mM), melatonin can induce HUVEC death by increasing the expression of p53 and Bax, and decreasing Bcl-2 [27]. However, the concentrations used in the present study did not exceed 100 μM. Furthermore, the sensitivity of human aortic endothelium to melatonin may differ from that of venous endothelium. The observed anti-nectrotic effect of melatonin observed in the present study was observed after the endothelium was damaged by 7-ketocholesterol, whereas the proapoptotic effects of melatonin on HUVECs were not associated with other stimulating factors.

The xCELLigence system allows for the monitoring of adhering cells. The lower absolute CI values observed in smooth muscle cells compared to HAECs may be attributed to the less tight cell junctions of smooth muscle cells. In the case of HuAoSMCs, the CI changes may be interpreted mainly as resulting from cellular apoptosis or proliferation processes. It was found that 100 μM melatonin decreased the cellular impedance of smooth muscle cells. While melatonin is not widely known to have an effect on the proliferation process of bovine aortic smooth muscle cells (AoSMCs) [32], it has been shown to prevent peroxynitrite-induced apoptosis of rat AoSMCs [33]. Additionally, Lee et al. demonstrated that melatonin induces mitochondrial energetic stress leading to the inhibition of vascular smooth muscle cell proliferation and apoptosis via stressinducible protein Sestrin 2, which regulates cell growth and survival [34]. However, flow cytometry analysis using Ax5-FITC and PI staining did not reveal any effect of melatonin on the viability of HuAoSMCs. The toxic effect of 7-ketocholesterol on smooth muscle cells, including HuAoSMCs, is not yet fully understood. The typical changes caused by 7-ketocholesterol in smooth muscle cells include reduced adhesion, modified actin organisation in the cytoskeleton, reduced transmembrane mitochondrial potential, and nucleus condensation [18, 35]. It has also been found to induce apoptosis in human smooth muscle cells isolated from the umbilical vein [17]. In addition, 7-ketocholesterol can lead to the accumulation of ubiquitinated proteins in the cytoplasm, potentially contributing to the instability of atheromatous plaque when acting on smooth muscle cells [36], which is associated with a higher risk of acute coronary syndromes [37].

The xCELLigence approach used in this study revealed that 7-ketocholesterol reduced cellular impedance, indicating a detrimental effect on HuAoSMCs. The results also demonstrate that 7-ketocholesterol increased the permeability of HuAoSMCs to PI and Ax5-FITC, indicating the induction of apoptosis. Melatonin administered before 7-ketocholesterol did not have an effect on the cellular death processes triggered by 7-ketocholesterol. Similarly, the xCELLigence system did not provide evidence of melatonin preventing the reduction in cellular impedance. Currently, no studies have determined whether melatonin is capable of protecting HuAoSMCs from the toxic effects of 7-ketocholesterol.

Although melatonin has been shown to prevent the apoptosis of rat AoSMCs caused by a strong oxidant like peroxynitrite [33], the results of the present study do not confirm whether melatonin can protect HuAoSMCs against the necrosis induced by 7-ketocholesterol. Melatonin has also been found to have a beneficial effect on the human vascular smooth muscle cell line CRL1999 when stimulated by lipopolysaccharide, as it inhibits the production of pro-inflammatory cytokines, inducible nitric oxide (NO) synthase, prostaglandin E2, and NO in a dose-dependent manner [38]. However, differences may be observed between this and other studies due to the model of melatonin used on primary AoSMC cells and the use of a different damaging agent. In another study, melatonin at high concentrations (1 mM), which potentially induces an antioxidative effect, failed to prevent 7-ketocholesterol-induced apoptosis and mitochondrial dysfunction in U937 cells [39].

CONCLUSION

This study provides confirmation of the toxic effect of 7-ketocholesterol on primary human vascular wall cells using 2 independent methods. The results demonstrate that melatonin does not have a barrier-protective effect. However, low concentrations of melatonin may counteract the toxic effects of 7-ketocholesterol on HAECs by inhibiting apoptosis. Melatonin does not have a significant effect on the impedance and viability of HuAoSMCs.

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