Research Article

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Modulation of CaMKII levels in Wharton's jelly mesenchymal stem cells under hydrogen peroxide induced stress conditions

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Abstract

Objectives: Calcium/Calmodulin-dependent protein kinase-2 (CaMKII) is a serine/threonine kinase prevalent in neuronal cells, playing a key role in memory, learning, and synaptic plasticity. Nonspecific CaMKII inhibition can prevent apoptosis in neuronal cells and reduce glutamate-induced cell death. Additionally, variations in CaMKK enzyme levels affect hemopoietic stem cell proliferation, although the effects of CaMKII on stem cell responses during stress remain unclear. This study aims to explore CaMKII's impact on the survival and proliferation of mesenchymal stem cells under stress and analyze the expression of its isoforms (alpha, beta, gamma, and delta) in these conditions.

Methods: The study included the characterization of MSCs, followed by an evaluation of the effects of KN-93, a CaMKII inhibitor, on the viability and proliferation of stem cells both in the presence and absence of H_2O_2 treatment. The toxicity caused by the application of 1 mM H_2O_2 further increased by inhibitor treatment. Additionally changes in the gene expression levels of CaMKII isoforms were analyzed.

Results: The application of H_2O_2 significantly decreased the total expression levels of CaMKII, with a significant reduction in the delta isoform. Furthermore, CaMKII inhibition by KN-93 increased the toxicity induced by H_2O_2. Viability and proliferation of stem cells were negatively impacted by the combined treatment of KN-93 and H_2O_2 compared to H_2O_2 alone.

Conclusions: Our findings provide a strong foundation to understand the response mechanisms of MSCs under stress conditions and could inform strategies for targeted stem cell therapies in oxidative stress conditions.

Keywords: mesenchymal stem cells; oxidative stress; Wharton's jelly; CaMKII; cell viability; KN93

Introduction

The term "kinase" encompasses a broad category of enzymes that facilitate a phosphate group transfer from ATP to Serine, Threonine, or Tyrosine hydroxyl groups in protein substrates [1]. The calcium/calmodulin-dependent protein kinase (CaM-kinase) family, including CaMKI, CaMKII, CaMKIII, CaMKIV, and CaMKK, consists of Serine/Threonine kinases activated primarily by the calcium (Ca^{2+})/calmodulin (CaM) complex. Some members can also undergo Ca^{2+}/CaM-independent activation through additional modifications, enabling precise regulation of diverse cellular functions [2]. The pursuit of a "memory molecule" led to significant findings, suggesting that these molecules could be kinases or phosphatases that act as switches for memory and cognitive processes [3]. Specifically, calcium/calmodulin-dependent protein kinase II (CaMKII) has garnered attention due to its pivotal role in learning and memory [4]. Beyond neuronal function, CaMKII is involved in regulating tumor cell

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survival and proliferation. Recent research has elucidated its integral role in autophagy and cellular responses to various stimuli [5, 6].

CaMKII is found in many tissues, but in higher concentrations in some parts of the brain, constituting as much as 2 % of the total protein especially in neuron cells [7]. CaMKII is abundantly present in brain tissues and throughout the body, playing a key role in coordinating Ca^{2+} signal transduction. Its phosphorylated substrates maintain cellular homeostasis and support activity-dependent neuronal modifications essential for learning and memory [8]. CaMKII holoenzymes have a unique structure, consisting of 12 functional domains arranged in a cogwheel-like shape with two clusters of six domains. Each subunit contains catalytic, autoregulatory, and helper domains. Binding of Ca^{2+}/CaM to the holoenzyme releases the autoregulatory inhibition, triggering autophosphorylation and resulting in changes such as Ca^{2+}/CaM-independent activity, CaM capture, and altered sensitivity. These mechanisms grant CaMKII a molecular memory capacity for its activity and autoregulation [9]. In mammals, CaMKII is encoded by four genes: α, β, γ, and δ isoenzymes. The α and β isoenzymes are more abundant in the brain. Each of these isoenzymes has many linkage variations. In invertebrates, a single alternatively folded gene encodes CaMKII [10]. It has been proposed that nonspecific inhibition of CaMKII can avert apoptosis in neuronal cells. Additionally, inhibiting both induced and autonomic CaMKII activity reduces glutamate-induced neuronal cell death in primary cultures [11].

The mechanism of CaMKII inhibition by KN-93 and its structural analog KN-92 involves the disruption of CaMKII's interaction with calmodulin (CaM), a process that is essential for its activation. KN-93 is a well-known inhibitor that specifically targets the CaMKII pathway, affecting various physiological processes. The inhibition of CaMKII by KN-93 has been shown to have significant effects on cellular functions, such as delaying ovarian follicle development [12] and increasing neuronal excitability [13], which are mediated through distinct molecular pathways. In contrast, KN-92 does not inhibit CaMKII, as it cannot interfere with the CaM-CaMKII interaction, thus lacking the physiological effects of KN-93. This highlights the specificity of KN-93 as a CaMKII inhibitor and the significant physiological changes resulting from its targeted inhibition [12].

Hydrogen peroxide (H_2O_2) is a crucial metabolite involved in various cellular processes and redox metabolism. It functions as a key secondary messenger, alongside hydrogen sulfide (H_2S) and nitric oxide (NO), triggering downstream protein cascades through specific oxidations. This response determines whether a cell proliferates, survives, or undergoes death, depending on the activated pathways (pathological, homeostatic, or protective) [14]. It is shown that H_2O_2 potently upregulates endothelial NO synthase (eNOS) gene expression in endothelial cells via activation of CaMKII and Janus kinase 2 (JAK2) [15]. Conversely, oxidative stress injury results in significant localized and widespread tissue damage, leading to functional failure in distant organs, such as cardiovascular system, the gastrointestinal tract, liver, and brain [16]. A study has demonstrated the function of 17b-estradiol (17b-E2) against oxidative stress on mice bone marrow mesenchymal stem cells (BMSCs) death triggered by hydrogen peroxide (H_2O_2) [17].

In the 1960s, stem cells first described in adult mouse bone marrow and they are categorized as embryonic, fetal or adult stem cells, according to the period of origin during ontogenesis [18]. Stem cells have the capacity to renew themselves over time, as they have a non-limited number of mitosis divisions and can be obtained both in the embryonic and in the adult period, and either prenatally or postnatally [19]. It has been shown that CaMKII is crucial in the bone marrow-derived mesenchymal stem cells chondrogenesis [20]. Another study has demonstrated a direct involvement of CaMKII in TGF-β and bone morphogenetic protein-mediated responses in chondrocytes derived from primary and pluripotent stem cells [21]. Although the specific interaction between WJ-MSCs and CaMKII is not directly addressed, CaMKII is known to regulate cellular signaling pathways involved in differentiation and proliferation, indicating its potential role in the neural differentiation of WJ-MSCs. The regulatory mechanisms of WJ-MSCs, including transcriptional and epigenetic modifications, may involve CaMKII, which is essential for maintaining pluripotency and guiding differentiation [22].

Additionally, recent research suggests that variations in the levels of Calcium/calmodulin-dependent protein kinase (CaMKK) enzyme, which belongs to the same family as CaMKII, regulate the proliferation of hematopoietic stem cells [23]. During embryogenesis and postnatal development, generating differentiated cells for tissue growth must balance stem cell continuity for future growth. This balance is maintained by initiating a proliferative phase that increases progenitor cell numbers before their differentiation into hypertrophic chondrocytes. The study found that increased CaMKII activity prevented hypertrophy, while loss of CaMKII function disrupted the transition from proliferation to hypertrophy [24]. It is known that the multifunctional CaMKII enzyme is involved in the phenotype change and maintenance of smooth muscle cells. In a study, it was determined that CaMKIIγ isoform plays a role in human adipose tissue-derived mesenchymal stem cell differentiation into contractile smooth muscle cells. Throughout the differentiation process, the level of CaMKIIγ gradually increased [25]. However, there remains a lack of clarity over

the effects of CaMKII and its isoenzymes' on cellular response in stem cell viability and proliferation in stressful conditions. In this study, we aimed to investigate the effect of CAMKII enzyme on the survival and proliferation of mesenchymal stem cells both with and without stress conditions, and also, to determine the alterations in gene expression and protein levels of CAMKII isoforms (alpha, beta, gamma, delta) under stress conditions. This study posits that inhibition of CaMKII will lead to a decrease in the survival and proliferation rates of mesenchymal stem cells, particularly under oxidative stress.

Materials and methods

Obtaining of umbilical cords, Wharton's jelly-derived MSCs isolation & culture and mesenchymal stem cell characterization

This study involved seven completely healthy pregnant women (aged 18–40) at term 37–40 weeks undergoing elective cesarean sections at Tepecik Obstetrics and Research Hospital, Izmir (For the study, ethical approval and informed written consent were obtained). Exclusion criteria included pregnancies from assisted reproductive techniques, fetal abnormalities, intrauterine growth retardation (fetal weight<2500 g), maternal disorders like diabetes or any other maternal risk factors. A 10–20 cm segment of the umbilical cord was collected, cleaned of excess blood, and preserved in +4 °C sterile phosphate buffer (PBS) containing 100 µg/ml streptomycin and 100 U/ml penicillin. The samples were then transferred to Dokuz Eylül University's Medical Biochemistry Department for further analysis.

The cleaned cord was dissected longitudinally using a sterile scalpel, removing the vessels, and the section containing the mucous layer (Wharton's jelly) was separated into 0.4–0.5 cm pieces (explant). These pieces were placed into 6-well plates. DMEM/F-12 medium supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin and 1 % L-glutamine was used to culture the cells and the plates were cultured in a 37 °C adjusted 5 % CO_2 incubator. Cell output from explants was observed after an average of 9–11 days. The medium of the cells was changed every three days for more efficient feeding and proliferation. Isolated cells were grown in plates for experiments or they were frozen at −80 °C until usage.

Isolated WJ-MSC's were characterized with Human Mesenchymal Stem Cell Functional Identification Kit (R&D Systems, Cat:SC006) like in our previous study [26]. In this study, the minimal criteria for characterizing Mesenchymal Stem Cells (MSCs), according to the International Society for Cellular Therapy (ISCT), were the ability to differentiate into adipogenic, osteogenic and chondrogenic cells. These criteria were demonstrated histochemically by the use of Wharton Jelly derived stem cells. The characterization of MSCs were isolated using explant method from Wharton jelly. During this process, multi lineage differentiation capacity and stemness-related cell surface antigen expressions were revealed by immunofluorescence microscopy, flow cytometry and q-PCR The reedited data from our previous study was shown in supplementary material [26].

Hydrogen peroxide (H_2O_2) toxicity, cell viability and proliferation assays

In this study, optimal doses of inhibitors and hydrogen peroxide (H_2O_2) were determined through preliminary experiments, focusing on viability, effect dose, and IC50 values. The study involved six experimental conditions: control, H_2O_2(Sigma, Cat:H1009) group, KN-93(BioVision, Cat:1909) inhibitor+H_2O_2, KN-93 inhibitor, KN-92(BioVision, Cat:B1643) negative inhibitor+H_2O_2, and KN-92 negative inhibitor. Cells (8×10^3) were cultured in 96-well plates and 200×10^3 cells in 6-well plates with complete DMEM/F-12 medium. After incubation at 37 °C in 5 % CO_2 for 16–18 h, cells were pre-treated with 1 µM KN-93 or KN-92 inhibitors for 24 h. Subsequently, 1 mM H_2O_2 was added, and plates were incubated for 30 min. All experiments were conducted a minimum of three times.

Thiazolyl Blue Tetrazolium Bromide (MTT) Kit (Applichem, Cat:A2231) was used to determine cell viability and CellTiter96® AQueous One Solution Cell Proliferation (MTS) Kit (Promega, Cat:G358) was utilized to assess cell proliferation. 8×10^3 cells were planted into 96-well cell culture plates with complete DMEM/F-12 cell culture medium at 200 µL final volume. All applications were performed as previously described, then the experiment was conducted.

Determination of specific gene expressions and measurement of CaMKII protein levels

The RT-PCR experiment analyzed surface antigen expressions and CaMKII isoforms (alpha-Qiagen:PPH02338A, beta-Qiagen:PPH01503E, gamma-Qiagen:PPH00525A, delta-Qiagen:PPH11180A) in WJ-MSCs from umbilical cords. Total RNA was isolated by using the MN Nucleospin II RNA isolation kit (Macherey-Nagel, Cat: 740-955.250) and RNA purity confirmed by Thermo NanoDrop 2000 device (A260/

A280 ~2). cDNA synthesis (2 µg) was done using the Qiagen RT2 First Strand Kit (Qiagen, Cat: 330404). qPCR was performed using specific primers and Qiagen RT2 SYBR Green Mastermix (Qiagen, Cat: 330600) on a LightCycler instrument. Expression of genes was standardized to GAPDH and quantified using the $2^{-(\Delta\Delta CT)}$ method. ΔCt was determined by subtracting GAPDH-CT from gene-CT, and relative expression was determined using control group data.

In this study, an ELISA experiment measured total CaMKII level in WJ-MSCs using Human CaMKII ELISA Kit (Fine Test, Cat: EH6990). Cells (200×10^3) were cultured in 6-well plates with DMEM/F-12 medium and incubated for 16–18 h at 37 °C in 5 % CO_2. Inhibitor and hydrogen peroxide treatments were applied as described previously. Cell supernatants and lysates were collected and ELISA experiments were performed and protein concentrations were determined by BCA assay (ThermoFisher Scientific, Cat:23225) according to the manufacturer's guidelines.

Statistical analysis

The data were analyzed using the SPSS Windows 25.0 software. Categorical variables were compared using the independent samples t-test, while continuous variables were assessed with the Mann-Whitney U test. Stem cells from the umbilical cords of seven pregnant women were utilized in the experiments, which were repeated between 3 and 6 times. Results are given as mean±standard error. $p<0.05$ values were considered statistically significant.

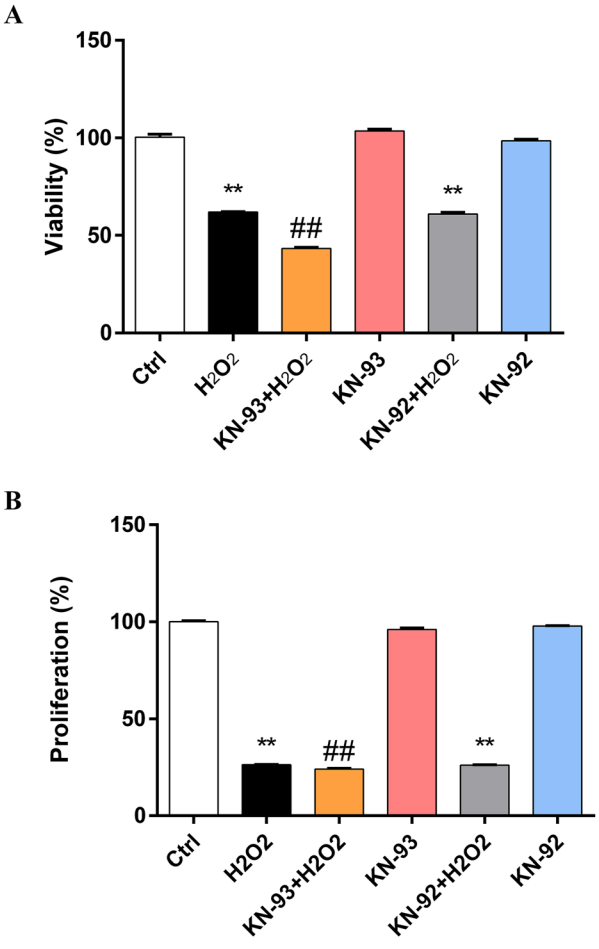

Figure 1: The effect of CaMKII inhibitor and negative inhibitor pre-application and H_2O_2 applications (A) on viability of WJ-MSCs and (B) on proliferation of WJ-MSCs.

Results

Cell viability and proliferation levels of WJ-MSCs

MTT results showed no significant change in cell viability with 1 µM KN-93 or KN-92 inhibitors applied for 24 h compared to the control. In the group treated with 1 mM H_2O_2 for 30 min, cell viability decreased by 38.1 %, and with 1 µM KN-92 pre-application, it decreased by 39 %, both statistically significant. After 1 µM KN-93 pre-application, H_2O_2 treatment reduced viability by 57 % in comparison to the control and 28 % compared to the KN-92+H_2O_2 and H_2O_2-only groups, also statistically significant (p=0.0079) (Figure 1A).

MTS test results showed no significant change in stem cell proliferation with 1 µM KN-93 or KN-92 applied for 24 h compared to the control. H_2O_2 (1 mM, 30 min) decreased proliferation by 73.7 %, and with 1 µM KN-92 pre-application 24 h, it decreased by 73.9 %, both statistically significant. With 1 µM KN-93 pre-application 24 h and H_2O_2 (1 mM, 30 min), proliferation decreased by 76 % compared to the control and 2.1 % compared to the H_2O_2 and KN-92+H_2O_2 groups, also statistically significant (p=0.0079) (Figure 1B).

Evaluation of CaMKII enzyme isoform levels by RT-qPCR

The results obtained by RT-qPCR method for CaMKII isoform changes for untreated-control WJ-MSCs show no statistically significant difference among the gene expressions of CaMKII alpha, beta, gamma and delta isoforms (p>0.05) (Figure 2).

CaMKII isoform changes were also examined in WJ-MSCs with H_2O_2 (1 mM, 30 min), showing decreased levels of CaMKII alpha, gamma and delta isoform gene expression levels relative to the control group, but increased CaMKII beta

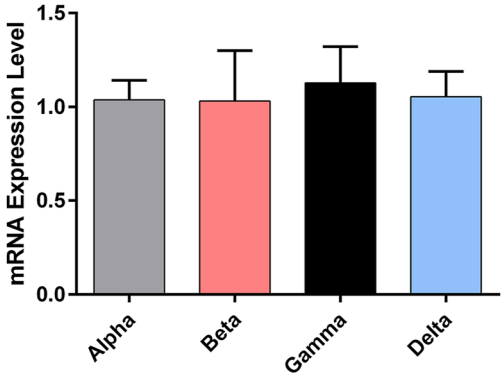

Figure 2: Gene expression levels of CaMKII *α*, *β*, *γ* and *δ* isoforms in WJ-MSCs without any treatment.

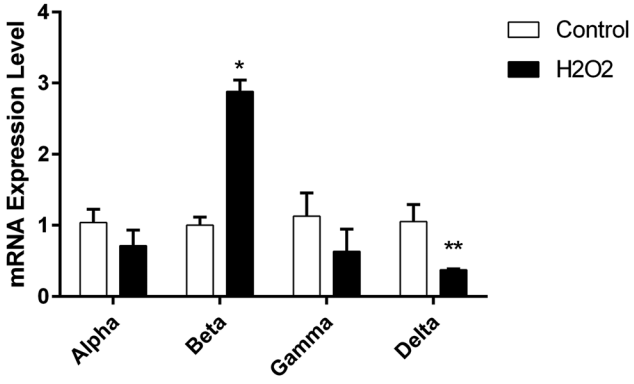

Figure 3: Gene expression levels of CaMKII *α*, *β*, *γ* and *δ* isoforms in WJ-MSCs under 1 mM H_2O_2 treatment for 30 min.

isoform gene expression level (p>0.05) (Figure 3) RT-qPCR results showed a decrease in gene expression in the 1 µM KN-93 and KN-92 application groups under 1 mM H_2O_2 stress, but it was not statistically significant (p>0.05). Also there was no statistically significant change in the KN-92 pre-treatment group in comparison to the control (p>0.05) (Figure 4). RT-qPCR results showed a 2.88-fold increase in CaMKII Beta gene expression under 1 mM H_2O_2 stress, and a 2.81-fold increase with 1 µM KN-92 pre-treatment. In the 1 µM KN-93 pre-treatment and H_2O_2 (1 mM, 30 min) group, expression decreased 0.5 times, and KN-93 alone reduced expression 8 times (p<0.05) (Figure 5).

The results showed a decrease in CaMKII Gamma gene expression in the 1 mM H_2O_2 stress group and the 1 µM KN-92 pre-treatment with H_2O_2 group, but the change was not statistically significant (p>0.05) (Figure 6). RT-qPCR data showed a significant 3-fold decrease in CaMKII Delta gene expression under 1 mM H_2O_2 stress (p=0.0079), and a 0.2-fold decrease with 1 µM KN-93 pre-applicaiton (p=0.0159). No notable changes were detected in CaMKII Gamma expression (Figure 7).

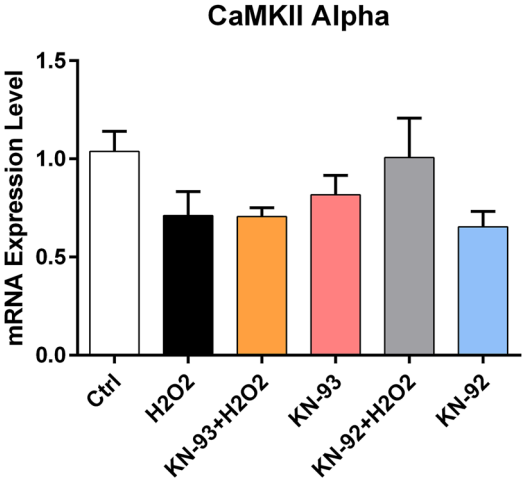

Figure 4: CaMKII alpha isoform gene expression change under CaMKII inhibitor and negative inhibitor pre-application and H_2O_2 applications in WJ-MSCs.

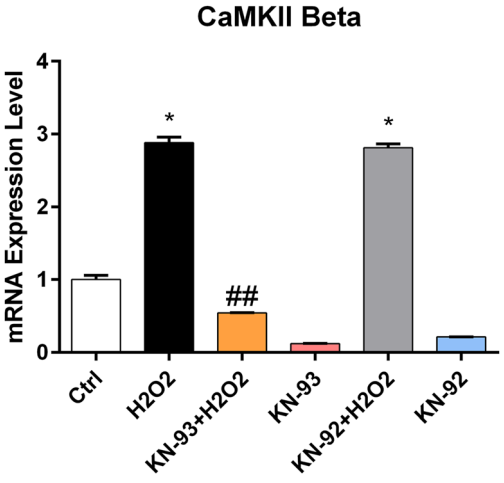

Figure 5: CaMKII beta isoform gene expression change under CaMKII inhibitor and negative inhibitor pre-application and H_2O_2 applications in WJ-MSCs.

Assessment of total CAMKII protein levels

A 64 % decline in total CAMKII protein level was observed in the 30 min 1 mM H_2O_2 stress condition in comparison to the control (p=0.0079). In the group in which 1 µM KN-92 pre-application for 24 h was applied and 1 mM H_2O_2 stress condition was applied for 30 min, a decrease of 62 % was found in comparison to the control group (p=0.0079). A decrease of 56 % was observed in the group in which 1 µM KN-93 CaMKII inhibitor pre-application for 24 h was applied and 1 mM H_2O_2 stress condition was applied for 30 min compared to the control group (p=0.0079) (Figure 8).

All results are presented as mean ± SEM.

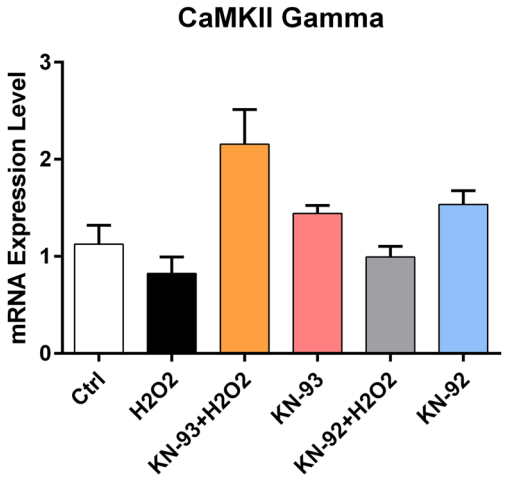

Figure 6: CaMKII gamma isoform gene expression change under CaMKII inhibitor and negative inhibitor pre-application and H_2O_2 applications in WJ-MSCs.

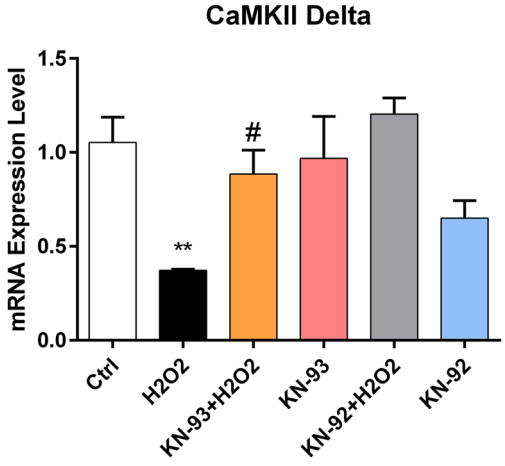

Figure 7: CaMKII delta isoform gene expression change under CaMKII inhibitor and negative inhibitor pre-application and H_2O_2 applications in WJ-MSCs.

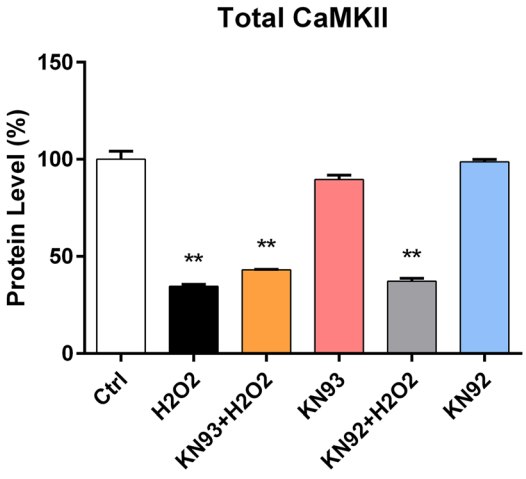

Figure 8: The effect of CaMKII inhibitor, negative inhibitor pre-application and H_2O_2 applications on total CaMKII protein levels in WJ-MSCs.

Discussion

This study aimed to address the limited understanding of CaMKII isoforms in regulating the survival and proliferation of mesenchymal stem cells (MSCs) under oxidative stress. While CaMKII is well-studied in neuronal systems, its role in MSCs, especially under stress, remains unclear. The study examined the effects of H_2O_2-induced oxidative stress and CaMKII inhibition on Wharton's Jelly-derived MSCs (WJ-MSCs). The results showed that oxidative stress significantly impaired WJ-MSC viability and proliferation, with KN-93-mediated CaMKII inhibition exacerbating these effects. Additionally, H_2O_2 exposure induced isoform-specific changes in CaMKII gene expression, including upregulation of the beta isoform and downregulation of the delta isoform. These findings highlight CaMKII's crucial role in MSC responses to oxidative stress and its potential as a target for improving MSC resilience in regenerative therapies.

The source and protocols for obtaining stem cells are essential for their potential in treating degenerative and proliferative diseases. While bone marrow stem cells are commonly used, MSCs from Wharton's Jelly possess stem cell characteristics and a reduced risk of tissue rejection due to the lack of associated proteins [27]. There has been growing interest in mesenchymal stem cells properties, particularly their ability to self-renewal and differentiation into various cell lines, and MSCs obtained from perinatal tissues are believed to possess greater differentiation potential than most adult MSCs [28]. Musiał-Wysocka et al. (2019) found that WJ-MSCs express pluripotency markers such as NANOG, OCT-4, and SSEA-4, but at lower levels than induced pluripotent stem cells (iPS), with expression potentially enhanced under hypoxic conditions. Notably, WJ-MSCs don't create tumors *in vivo*, highlighting their promise in regenerative medicine [29].

Limited information exists on protocols for extracting stem cells from Wharton's jelly and their stress responses. This study isolated MSCs from Wharton's Jelly under laboratory conditions, revealing minimal stem cell properties [26]. These cells were examined, and their characteristics were also identified in the current study. Experiments showed that oxidative stress from H_2O_2 reduced cell viability and proliferation by approximately 40–50 % compared to the control group. The optimal dose and duration for WJ-MSCs were determined based on the dose-response

curves for the KN-93 CaMKII inhibitor and the KN-92 negative inhibitor. Doses of 5 and 10 μM of KN-93 decreased cell viability and were excluded. Given that the IC50 for KN-93 is 0.37 μM, a 1 μM dose, which had no negative effects on cell viability, was used in all experiments. This dose has also been employed in similar studies for 24 h with both inhibitors [30]. This study assessed the effects of the KN-93 CaMKII inhibitor on WJ-MSC viability, proliferation, and changes in gene expression of CaMKII isoforms and total CaMKII protein levels under H_2O_2-induced stress. The results showed that combining KN-93 with H_2O_2 significantly reduced cell viability and proliferation compared to both the H_2O_2-stressed and control groups, highlighting the crucial role of CaMKII in these processes. This supports the hypothesis that "the living cell learns, and the learning cell lives," emphasizing CaMKII's role as an enzyme in learning.

One study previously showed that CaMKII has no impact on BMSCs proliferation, but can hinder their chondrogenic potential by impacting their differentiation [20]. Another study showed that the CaMKII mRNA levels were significantly upregulated when treated with H_2O_2 in BMSCs [31]. In WJ-MSCs, the application of H_2O_2 stress significantly increased the gene expression of the CaMKII Beta isoform but returned to control levels in both the KN-93 and H_2O_2 applied groups. H_2O_2 was shown to activate CaMKII in astrocytes, and this activation can be blocked by KN-93 [32]. H_2O_2 stress increased CaMKII Beta isoform gene expression, suggesting the enzyme's role in maintaining cell viability and proliferation. Additionally, H_2O_2 application increased CaMKII activity due to methionine oxidation effect of H_2O_2 itself [33]. An increase in CaMKII Beta isoform was observed in groups treated with both KN-92 and H_2O_2, as well as in the H_2O_2-only group, indicating that KN-93 significantly affected its expression. A statistically significant decline in CaMKII Delta isoform was found only in the H_2O_2 stressed group compared to controls, with levels approaching control in the KN-93 and H_2O_2 combination group. No significant changes were observed for CaMKII Alpha and Gamma isoforms.

CaMKII was initially discovered in the brain, and it is regarded as a key target for cerebral ischemic nerve injury; phosphorylated CaMKII translocates from the cytoplasm to the cell membrane [34]. The total flavonoid extract of *Dracocephalum moldavica* L. (TFDM) has been shown to protect astrocytes against H_2O_2-induced apoptosis by attenuating a CaMKII-dependent mitochondria pathway [35]. Another study in mesenchymal stem cells has demonstrated that Ganoderic Acid D (GA-D) prevented oxidative stress induced senescence by the activation of CaM/CaMKII/NRF2 signaling pathway [36]. In our study, total CaMKII protein levels significantly decreased under both H_2O_2 stress and H_2O_2 stress with KN-92 pre-application conditions. Furthermore, H_2O_2 stress with KN-93 CaMKII inhibitor caused a dramatic decrease compared to controls, likely due to rapid degeneration of phosphorylated proteins. While phosphorylated forms were not assessed in our ELISA tests, they showed an increase compared to the H_2O_2-only group and a slight increase relative to the KN-92+H_2O_2 group. Although H_2O_2 typically increases CaMKII activity, our findings suggest that hydrogen peroxide-induced cytotoxicity led to a decrease in the enzyme's protein level. This reduction may have triggered an increase in gene expression, particularly of the beta isoform, as a cellular response.

The latest findings indicate that each of the four isoforms serves distinct functions and in some cases, these roles is entirely independent of their enzymatic function. Recent research highlights the Delta isoform's crucial role in memory maintenance and persistence through the sustained expression of its gene [37]. Also, it has been shown that in rats, CaMKII-delta protein expression increase for as long as 5 days after brain injury [38] and as long as 7 days in homogenates form ventricles following transverse aortic constriction [39]. In our study, H_2O_2 application significantly decreased the total CaMKII expression level, especially for the delta isoform. The detection of CaMKII Delta in both presynapses and the nucleus is a key finding that deserves further investigation.

Conclusions

This study represents the first to explore and demonstrate the critical role of CaMKII in regulating the viability of mesenchymal stem cells (MSCs) under oxidative stress conditions induced by H_2O_2. CaMKII is integral to multiple signaling pathways that control cell survival, proliferation, and differentiation. A deeper understanding of its function offers valuable insights into the mechanisms through which MSCs sustain their effectiveness under stressful environments, thereby supporting their regenerative potential. Notably, CaMKII acts as a molecular memory mechanism, allowing cells to retain and adapt to previous signaling events. This capability enhances the adaptability and long-term functionality of MSCs under adverse conditions. Additionally, CaMKII plays a significant role in modulating cellular responses to oxidative stress, a hallmark of many pathophysiological processes. Understanding the specific impact of CaMKII on MSC behavior during oxidative stress can inform the development of targeted strategies to improve MSC resilience and effectiveness in regenerative medicine applications.

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Research ethics: This study was approved by the Dokuz Eylul University Non-Interventional Research Ethics Committee with the 4061-GOA protocol and decision number 2019/08–35 on 03.04.2019. Informed written consent was obtained from all women included in this study.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

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