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Original Research Article

Tunicamycin induced inhibition of calpain 1 and 2 enzyme activity in ovarian cancer cells

Seda M. Sari Kilicaslan[1]*, Zerrin İncesu[2]

[1]Faculty of Education, Anadolu University, Eskisehir, Turkey
[2]Department of Biochemistry Science, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

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***Correspondence:**
Dr. Seda M. Sari Kilicaslan,
Email: smskilicaslan@anadolu.edu.tr

ABSTRACT

Background: Tunicamycin (TN) is an antitumor agent and induced intracellular calcium levels in many cells, however its molecular mechanism is still needed to be explored. Calpeptin (Calp) is an inhibitor of both calpain 1 and 2 (CAPN-1/2) enzymes, and plays a fundamental role in tumor mechanism. In this study, the effects of TN and Calp were investigated on CAPN-1/2 enzyme activity in normal and ovarian cancer cells adhered to fibronectin.
Methods: 24uM TN, 50uM Calp, and combined TN and Calp (TN+Calp) were applied for 1 and 12 hours to FN-bound (FN+) and non-FN-bound (FN-) normal human ovarian epithelial (IHOSE) and ovarian cancer (SKOV-3) cells. The activation of CAPN-1/2 was measured by the luminescent method and the significance of the results was analyzed with the t-test.
Results: CAPN-1/2 enzyme activity (at 12 hour) was present in both cell lines, but the level of enzyme is higher in IHOSE cells compared to SKOV-3 cells. The results showed that 1 hour TN and TN+Calp applications stimulated CAPN-1/2 enzyme activity in IHOSE cells but did not show any stimulating effect in SKOV-3 cells. After 12-hour of treatment, the cells with TN, Calp or TN+Calp showed an inhibitory effect on CAPN-1/2 enzyme in both FN+IHOSE and SKOV-3 cells. At 12-hour TN+Calp administration was determined to be the most effective inhibitor in FN+ SKOV-3 since it inhibited CAPN1/2 activity statistically significantly more than both Calp and TN administrations.
Conclusions: The effects of TN, Calp and TN+Calp applications on the CAPN-1/2 enzyme varied according to the cell type, normal or cancer cells, and whether the cell was bound to FN and the incubation period. 12 h administrations of TN, Calp or TN+Calp inhibited the CAPN-1/2 enzyme in both FN+ IHOSE and SKOV-3 cells.

Keywords: IHOSE, SKOV-3, Fibronectin, Calpain

INTRODUCTION

Calpains are a family of intracellular calcium-activated neutral cysteine prostheses and expressed in many organisms. Calpain affects cell physiology in many ways such as cell attachment, proliferation, migration, death, cytoskeletal remodeling, and signal transduction, by degrading their substrates, providing the formation of functional protein fragments. The best known calpain are CAPN-1 and 2 so-called micromolar (calpain-1, CAPN-1) and millimolar (calpain-2, CAPN-2) depending on the Ca^{2+} requirements required for their proteolytic activity.[1]

In the normal physiological state, calpain enzymes and calpastatin, an endogenous calpain inhibitor, molecules are at normal levels. Calpain activity, on the other hand, may increase and cause proteolysis of calpastatin due to a stimulus such as increased intracellular Ca^{2+}, ischemia damage, or activation of v-Src oncoprotein.[2]

Calpains are involved in tumor migration and invasion by participating in processes such as focal adhesion dynamics, cytoskeletal remodeling, epithelial-mesenchymal transition, and apoptosis.[3] In studies on ovarian cancer, increased calpain activity has been found

to be associated with ovarian cancer and metastasis.[4] In addition, CAPN-2 expression was found to be increased in platinum resistance in ovarian cancer.[5] Contrary to these studies, in some studies, it was reported that the expression of CAPN-1, CAPN-2, and calpastatin decreased in gynecological cancer.[6] In a study conducted in vitro, it was noted that the protein expression of CAPN-1, 2 and calpastatin varies according to the cell type.[7] Therefore, the role of the calpain family in ovarian cancer is still a controversial issue and more research is needed. Based on the studies showing that inhibition of calpain has the potential to reduce carcinogenesis and block the metastasis of tumors, calpain has been shown as a therapeutic target in cancer.[8]

Tunicamycin (TN) activates Ca^{2+} from intracellular stores and increases Ca^{2+} flow.[9] TN is an antibiotic that promotes apoptosis by inducing stress in the endoplasmic reticulum and is thought to work as a therapeutic agent against cancer cells.[10] Therefore, in recent years, there has been an interest in the molecular mechanism of TN in cancer. It is known that fibronectin (FN) protein, an extracellular matrix protein, is effective during the migration of ovarian cancer cells.[11] In this study, FN-bound (FN+) and non-FN-bound (FN-) normal ovarian epithelial IHOSE and human ovarian cancer SKOV-3 cells were used. The effects of stimulating Ca^{2+} increase with TN and applying the Calpeptin (Calp), CAPN-1/2 enzyme inhibitor, to the cells on CAPN-1/2 enzyme activity. The obtained results will contribute the explanation of the functions of both calpains and TN in ovarian cancer.

METHODS

Cell culture

IHOSE

Immortalized human ovarian epithelial cells (IHOSE-SV40) (ABMGOOD T1074) were used as the control cell line. The cells were cultured into Prigrow I solution (ABMGOOD TM001), 10% foetal bovine serum (FBS) (HyClone SV3016-03), and 1% penicillin-streptomycin (P4333).

SKOV-3

Human ovarian cancer cells SKOV-3 (ATCC® HTB-77™) were used as a cancer cell line. The cells were cultured in DMEM (Sigma D6429), 10% FBS, 1% penicillin-streptomycin (P4333), 0.1 mM MEM non-essential amino acids (Sigma Aldrich, UK).

Fibronectin coating process

In order to coat FN, the petri dishes were incubated with 50 µg/ml FN at 37°C for 2 hours, followed by 1% bovine serum albumin (BSA) for 1 hour at 37°C, then washed 2 times with 1×PBS.[11]

Tunicamycin application

TN activates Ca^{2+} from intracellular stores and increases Ca^{2+} flow.[9] In this study, 24 µM TN (T7765, Sigma, UK) was added to the cells and incubated at 37°C for 1 hour or 12-hour.

Calpeptin application

Cell-permeable calpeptin (sc-202516) was used for the inhibition of CAPN-1/2 in the experiments. In the experimental phase, 50 µM calpain inhibitor was applied both alone and together with 24 µM TN and incubated for 1 hour and 12-hour.

Calpain activity measurement

Activity measurement of CAPN-1/2 was performed with the Calpain-GloTm Protease (G8501, Progema) kit as directed by the manufacturer. To measure CAPN-1/2 enzyme activity, 1×10^5 cells/ml IHOSE or SKOV-3 cells were seeded in FN-coated and uncoated petri dishes and incubated at 37°C for 24 hours for CAPN-1/2 enzyme activity. 24 µM TN, 50 µM Calp, and the combined administration (24 µM TN and 50 µM Calp) were added to the cells 1 hour /12 hour before the end of the incubation period. After the incubation period was ended, the cells were washed with a cold PBS solution and lysed by using sonication. Calpain-GloTm solution, Suc-LLVY-Glo substrate, and Luciferin Recognition Reagent were added to the obtained lysates. With the activation of CAPN-1/2, the substrate Suc-LLVY-aminoluciferin is decomposed by the luciferase reaction, creating a luminescent signal. The amount of signal formed is proportional to the current calpain activity. Afterward, end-point measurement was performed between 5-60 minutes with a luminance change microplate luminometer. Measurement results obtained at 60 minutes were evaluated using the t-test.[12] Human CAPN- I enzyme (Calbiochem Cat. No 208713) was used as the control.

Statistical analysis

The results of calpain activation obtained were evaluated using student's t-test (***$p \leq 0.01$; **$p \leq 0.05$; *$p \leq 0.10$).

RESULTS

CAPN-1/2 enzyme activity in IHOSE and SKOV-3 cells

CAPN-1/2 enzyme activity in FN+/FN- normal IHOSE and cancer SKOV-3 cells was measured luminometrically. The statistical significance of the results was checked by carrying out a t-test (Figure 1). CAPN-1/2 enzyme activity was found to be higher in FN+ IHOSE cells compared to SKOV-3 cells both at 1 hour and 12-hour. While this result was not statistically significant at 1 hour, it was found to be significant at 12-hour. In the FN- group, CAPN-1/2 enzyme activity was observed more in SKOV-3 cells at 1 hour and in IHOSE cells at 12-hour (Figure 1).

Effect of Calp and TN application on CAPN-1/2 enzyme activity

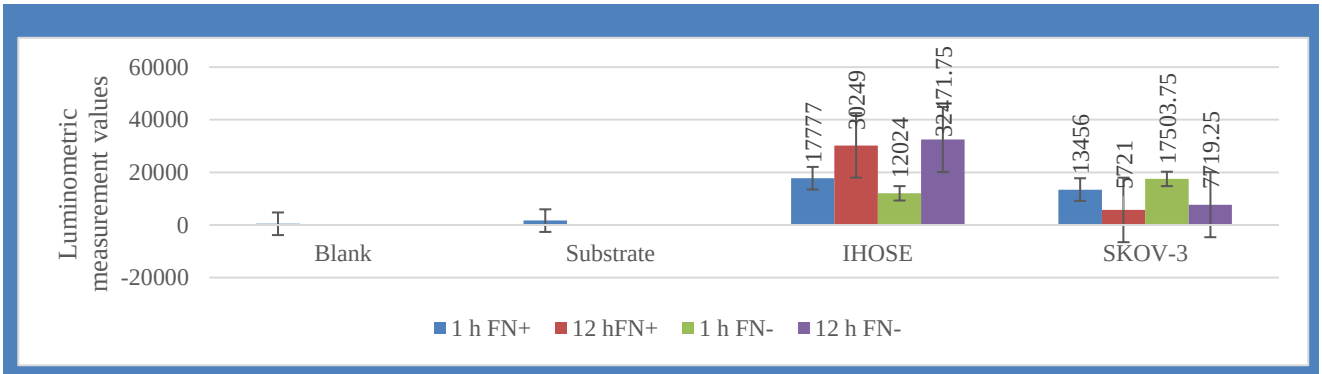
The effects of Calp, TN, and TN+Calp for 1 hour and 12-hour applications on CAPN-1/2 enzyme activity in FN+/FN- IHOSE cells were measured (Figure 2). Calp administration did not cause a significant effect on CAPN-1/2 enzyme activity in FN+/FN- normal IHOSE cells at 1 hour but caused a significant inhibition at 12 hours. TN administration induced the activation of CAPN-1/2 in FN+/FN- IHOSE cells at 1 hour compared to the control, while it led to inhibition at 12-hour. The effect of combined TN+Calp application on CAPN-1/2 enzyme activity for 1 hour did not cause a significant change in the FN- group compared to the control group, while it caused a significant increase in FN+ IHOSE cells. This result can be explained by the stimulating effect of TN on CAPN-1/2 activity in 1 hour in the FN+ group. At 12 hours, TN+Calp significantly inhibited the CAPN-1/2 enzyme in the FN+/FN- groups compared to the control group. In addition, TN+Calp administration inhibited CAPN-1/2 enzyme activity significantly more than TN administration in all groups (1 h FN+, 12 h FN+, 12 h FN-) except for 1hour FN- group. In FN+ IHOSE cells, TN+Calp administration significantly increased CAPN-1/2 enzyme activity compared to Calp administration alone in 1 hour but diminished in 12-hour.

This result, however, was not statistically significant. In the FN- group, TN+Calp application increased the CAPN-1/2 enzyme activity compared to Calp application in 1 hour, but it was not found statistically significant. On the other hand, the TN+Calp application caused a significant decrease in 12-hour. When the effect of FN on CAPN-1/2 enzyme activity in IHOSE cells was examined, no significant difference was found in CAPN-1/2 enzyme activity between FN+ control and FN- control groups. However, CAPN-1/2 enzyme activity was found to be significantly lower in FN- groups compared to FN+ groups in 1 hour TN, Calp, TN+Calp administration and 12-hour TN+Calp administration (Figure 2).

The result of the application of TN, Calp, and combined TN+Calp to FN+/FN- SKOV-3 cells for 1 and 12 hours on the activation of the enzyme of CAPN-1/2 was presented in Figure 3.

Calp administration for 1 hour significantly inhibited the CAPN-1/2 enzyme in FN+ SKOV-3 cells compared to the control cell group. 12-hour of Calp administration significantly inhibited CAPN-1/2 enzyme activity in FN+/FN- SKOV-3 cells similar to the result in IHOSE cells. 1 hour TN administration did not show any significant change in FN+ SKOV-3 cells but showed an inhibitory effect on FN- SKOV-3 cells. After 12-hour of TN application, there was significant inhibition in FN+ SKOV-3 cells, but no significant change was observed in the FN- group. TN+Calp administration caused significant inhibition of CAPN-1/2 enzyme activation at both 1 and 12 hours in FN+/FN- SKOV-3 cells compared to the control cell group. In FN+ SKOV-3 cells, 12-hour of TN+Calp administration inhibited CAPN1/2 activation statistically significantly more than both Calp and TN administrations. 1 hour TN+Calp administration in FN- SKOV-3 cells caused a significant increase compared to the Calp group.

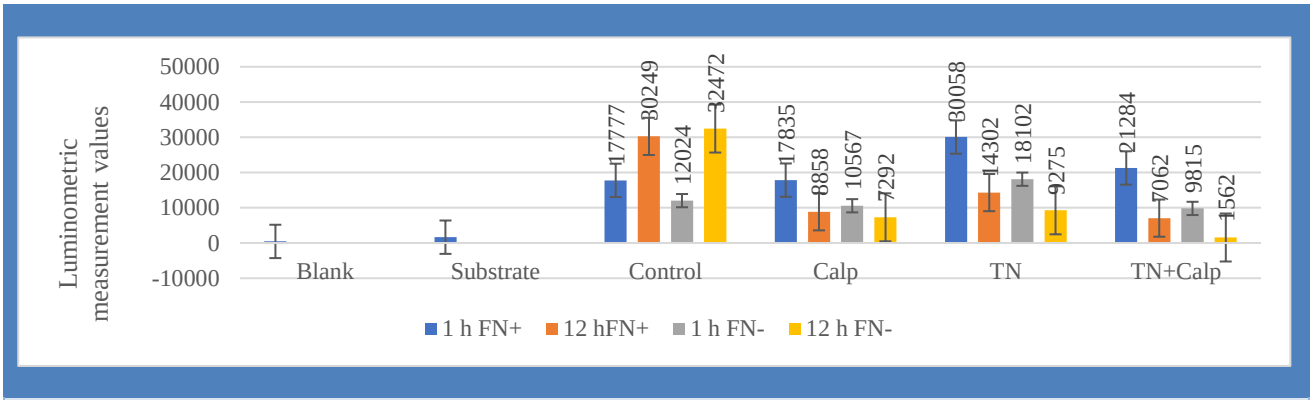
The results of the effect of FN on CAPN-1/2 enzyme activity in SKOV-3 cells show a significant difference between FN+ SKOV-3 and FN- SKOV-3 control cells at 1 hour, but not at 12-hour. A statistically significant difference was observed between FN+ and FN- groups at 12-hour only in the TN+Calp group. CAPN-1/2 enzyme was found to be significantly lower in the FN+ group compared to the FN- group in TN +Calp application in SKOV-3 cells.



1 h FN+: IHOSE=SKOV-3 insignificant | 12 h FN+: IHOSE=SKOV-3: *** | 1 h FN-: IHOSE = SKOV-3: ** | 12 h FN-: IHOSE=SKOV-3: ***

Figure 1: CAPN-1/2 enzyme activity after 1 and 12 hours' incubation of FN+/FN- IHOSE and SKOV-3 cells.

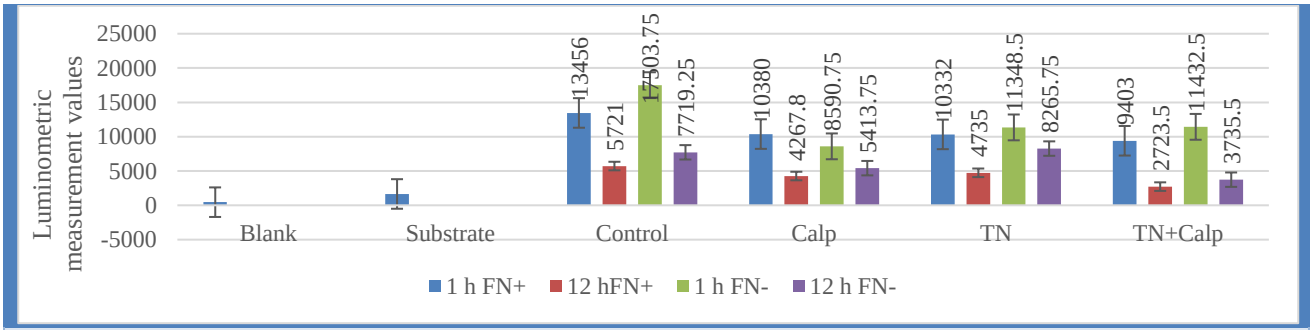
The luminometric values in the graph were calculated by taking the mean of two independent experiments (n=4). 1 h FN+: The cells incubated for 1 h in FN-coated petri dishes, 12 h FN+: The cells incubated for 12 h in FN-coated petri dishes, 1 h FN-: The cells incubated for 1 h in FN uncoated petri dishes, 12 h FN-: The cells incubated for 12 h in FN uncoated petri dishes (***p≤0.01, **p≤0.05, *p≤0.10)



1 h FN+	12 h FN+	1 h FN-	12 h FN-	1 h FN + and 1 h FN-	12 h FN + and 12 h FN-
Control=TN:**	Control=Calp:***	Control=TN:***	Control=Calp: ***	Calp=Calp:**	TN+Calp=TN+Calp:**
Control=TN+Calp:**	Control=TN:***		Control=Tn:***	TN=TN:***	
Calp=TN+Calp:*	Control=TN+Calp:***		Control=TN+Calp:***	TN+Calp=TN+Calp:***	
TN= TN+Calp:**	TN=TN+Calp:**		Calp=TN+Calp:***		
			TN=TN+Calp:***		

Figure 2: The effect of 1 h and 12 h administration of TN, Calp, TN+Calp on CAPN-1/2 enzyme activity in FN+/FN- IHOSE cells.

The luminometric values in the graph were calculated by taking the mean of two independent experiments (n=4). 1 h FN+: The cells incubated for 1 h in FN-coated petri dishes,12 h FN+: The cells incubated for 12 h in FN-coated petri dishes, 1 h FN-: The cells incubated for 1 h in FN uncoated petri dishes, 12 h FN-: The cells incubated for 12 h in FN uncoated petri dishes. Substrate: 22,5 nM CAPN-1/2 enzyme, TN: The group incubated with 24 µM TN. Calp: The group incubated with 50 µM Calp, TN+Calp: the group incubated with 24 µM TN and 50 µM Calp. (***p≤0.01, **p≤0.05, *p≤0.10)



1 h FN+	12 h FN+	1 h FN-	12 h FN-	1 h FN + and 1 h FN-	12 h FN + and 12 h FN-
Control=Calp:**	Control=Calp:***	Control=TN**	Control=Calp:***	Control=Control:**	TN+Calp=TN+Calp:**
Control=TN+Calp:*	Control=TN:*	Control=TN+Calp:**	Control=TN+Calp:*		
	Control=TN+Calp:***	Calp=TN+Calp:*			
	Calp=TN+Calp:***				
	TN=TN+Calp:**				

Figure 3: The effect of 1 h and 12 h administration of TN, Calp, TN+Calp on CAPN-1/2 enzyme activity in FN+/FN- SKOV-3 cells.

The luminometric values in the graph were calculated by averaging two independent experiments (n=4). 1 h FN+: The cells incubated for 1 h in FN-coated petri dishes, 12 h FN+: The cells incubated for 12 h in FN-coated petri dishes, 1 h FN-: The cells incubated for 1 h in FN uncoated petri dishes, 12 h FN-: The cells incubated for 12 h in FN uncoated petri dishes. Substrate: 22,5 nM CAPN-1/2 enzyme, TN: the group incubated with 24 µM TN. Calp: The group incubated with 50 µM Calp, TN+Calp: the group incubated with 24 µM TN and 50 µM Calp (***p≤0.01, **p≤0.05, *p≤0.10)

As a result, CAPN-1/2 enzyme activity was determined in both cell lines, which was higher in FN+ IHOSE cells than in FN+ SKOV-3 cells after 12-hour of incubation. While 50 µM Calp application for 1 hour was sufficient to inhibit CAPN-1/2 enzyme activity in FN+ SKOV-3 cells, it was determined that it was not sufficient in FN+/FN- IHOSE cells. While TN administration for 1 hour had a stimulating effect on the activation of CAPN-1/2 in FN+/FN- IHOSE cells, no stimulating effect was observed in SKOV-3 cells. In addition, 1 hour TN+Calp application showed a stimulating effect on CAPN-1/2 enzyme activity in FN+ IHOSE cells, while it showed an inhibitory effect on FN+ SKOV-3 cells. TN application for 12-hour inhibited CAPN-1/2 enzyme activity in FN+/FN- IHOSE cells and in FN+ SKOV-3 cells. 12-hour of Calp and TN+Calp treatments inhibited CAPN-1/2 enzyme activity in both cell lines in FN+ and FN- groups. TN+Calp administration for 12-hour in FN+ SKOV-3 cells inhibited CAPN1/2 enzyme activation statistically significantly more than both Calp and TN groups. In addition, TN+Calp administration in SKOV-3 cells had a stronger inhibitory effect in fibronectin-bound cells than in non-bound cells. On the contrary, in IHOSE cells, it showed stronger inhibition in cells not bound to fibronectin.

DISCUSSION

Ca^{2+}-dependent calpains, included in the protease family, have roles in multiple physiological and pathological functions. It is known that activation or inhibition of these protease family members is observed in many metabolic disorders such as neurodegenerative and cardiovascular diseases, ischemic disorders, arterial sclerosis, and cancer.[13] It has been reported that CAPN-1/2 is required for the invasive and metastatic potential of CAPN-1/2 in hepatocellular carcinoma.[14] In our study, we first demonstrated the presence of CAPN-1/2 enzyme activation in FN+/FN- normal IHOSE and ovarian cancer SKOV-3 cells. It is known that FN binding protein is effective during the migration of ovarian cancer cells *in vivo.*[11] It has been reported that calpain plays an important role in the FN induced EMT response in breast cancer.[15] Therefore, we conducted our research in FN+ and FN- cells and examined the effect of FN on CAPN-1/2 enzyme activation. CAPN-1/2 enzyme activity was found to be significantly higher in FN- SKOV-3 cells compared to FN+ cells in 1 hour of application. No significant difference was observed in FN+ and FN- control groups at 12-hour. Kondakova et al in their study on calpain activity, found calpain activity to be significantly higher in ovarian cancer compared to normal tissue and in metastatic ovarian cancer compared to primary tumors.[4] In patients with primary ovarian cancer treated with platinum-based chemotherapy, CAPN-1 and CAPN-2 expressions were detected and a high level of CAPN-2 expression was significantly associated with platinum-resistant tumors.[5] In an *in vitro* study, CAPN-1, 2 protein expressions were investigated in 5 different ovarian cancer cells (A2780, A-2780-cis, SKOV-3, PEO1, PEO4) and shown that CAPN-1 expression was highest in PEO1 cells and CAPN-2 expression in SKOV-3 cells. The same study reports that the expression of these proteins is very low in some types of cells (A2780, A2780cis), and it varies according to the cell type.[7] In our study, we found that CAPN-1/2 enzyme activity (12-hour) was higher in FN+ normal IHOSE cells compared to SKOV-3 cells. On the other hand, it was more expressed in SKOV-3 cells in FN- cells after 1 hour of incubation, while enzyme activity was observed to be higher in IHOSE cells with the prolongation of the time (12 h). As a result, in our study, we can say that the activity of CAPN-1/2 varies according to the cell, the presence of FN, and the incubation period.

TN, a nucleoside antibiotic, activates Ca^{2+} from intracellular stores, and increases Ca^{2+} flux through the plasma membrane.[9] A recent in vivo study found that TN treatment activates both cytosolic and mitochondrial calpain 1 (CAPN-1).[16] In a study investigating the anti-tumor effect of TN, it was also found that TN inhibits cell proliferation and migration in hepatocellular carcinoma.[17] In our study, we examined the effect of TN on CAPN-1/2 activation in FN+/FN- normal and cancer cells. The effect of TN on the CAPN-1/2 enzyme activity varied according to both the cell type, whether the cells were FN bound or not, and the duration of application. While TN stimulated CAPN-1/2 enzyme activity in FN+/FN- normal IHOSE cells at 1 hour, it did not show any stimulating effect on SKOV-3 cells. We can say that TN application for 12-hour acts as a CAPN-1/2 inhibitor because it significantly inhibits CAPN-1/2 enzyme activity in FN+ SKOV-3 and IHOSE cells. It is promising that calpains can be used as a target molecule in cancer treatment and that specific inhibitory substances for clinical use can be developed and applied in the treatment. Rose et al showed that the CAPN-2 inhibitor substance (2LLY-CH2F) reduced tumor volume in murine colitis and colitis-related cancers and reduced HT29 human colorectal cancer cell proliferation.[18] Proliferation and migration were inhibited in high-grade serous ovarian cancer by inhibiting CAPN9 gene expression with MiR-585-3p.[19] In our research, we determined the effect of Calp application on CAPN-1/2 enzyme activity in both normal and cancer cells. Calp administration 1 hour significantly inhibited CAPN-1/2 activation in FN+ SKOV-3 cells, while it did not show an inhibitory effect in other groups (FN- SKOV-3 and FN+/FN- IHOSE). On the other hand, 12-hour of Calp application inhibited CAPN-1/2 activation in both IHOSE and SKOV-3 cells, independent of FN, at a statistically significant level. However, both TN and Calp showed an inhibitory effect on FN+ IHOSE and SKOV-3 cells after 12-hour of administration. It has been determined that when TN+Calp is applied to FN+ SKOV-3 cells for 12-hour, it inhibits CAPN-1/2 enzyme activity more strongly than TN and Calp applications alone. In addition, TN+Calp administration inhibited FN+ SKOV-3 cells more than FN.

CONCLUSION

In this study, the effect of TN, which provides cytoplasmic calcium increase, and CAPN-1/2 inhibitor, Calp, on

CAPN-1/2 enzyme activity in FN+ ovarian cancer cells was explored by comparing with normal ovarian cells. CAPN-1/2 enzyme activity was demonstrated in both normal and SKOV-3 cancer cell lines, being higher in normal IHOSE cells (12 h). TN and TN+Calp administrations for 1 hour stimulated calcium-activated CAPN-1/2 enzyme activity in FN+ IHOSE cells, while they did not show any stimulatory effect in SKOV-3 cells. In fact, 1 hour TN+Calp administration showed an inhibitory effect on CAPN-1/2 enzyme activity in FN+ SKOV-3 cells. TN, Calp and TN+Calp administration for 12-hour inhibited CAPN-1/2 enzyme activity in FN+/FN- IHOSE and SKOV-3 cells. TN+Calp application was determined to be the strongest inhibitor in FN+ SKOV-3 cells since it inhibited more than both Calp and TN alone. In addition, TN+Calp administration in SKOV-3 cells had a stronger inhibitory effect in fibronectin-bound cells than in non-bound cells. In conclusion, the effects of TN, Calp and TN+Calp applications on the CAPN-1/2 enzyme varied according to the cell whether it was bound to FN or not and the incubation period. 12 h administrations of TN, Calp or TN+Calp inhibited the CAPN-1/2 enzyme in both FN+ IHOSE and SKOV-3 cells.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Kovacs L, Su Y. The Critical Role of Calpain in Cell Proliferation. J Biomol Res Ther. 2014;3(3):1000112.
2. Carragher ON, Frame CM. Calpain: a role in cell transformation and migration. Int J of Biochem Cell Biol. 2002;34:1539-43.
3. Chen J, Wu Y, Zhang L, Fang X, Hu X. Evidence for calpains in cancer metastasis. J Cell Physiol. 2019;234(6):8233-40.
4. Kondakova IV, Yunusova NV, Spirina LV, Kolomiets LA, Villert AB. Association between intracellular proteinase activities and the content of locomotor proteins in tissues of primary tumors and metastases of ovarian cancer. Russ J Bioorg Chem. 2014;40:681-7.
5. Storr SJ, Safuan S, Woolston CM, Abdel-Fatah T, Deen S, Chan SY, Martin SG. Calpain-2 expression is associated with response to platinum based chemotherapy, progression-free and overall survival in ovarian cancer. J Cellular Mol Med. 2012;16:2422-8.
6. Salehin D, Fromberg I, Haugk C, Dohmen B, Georg T, Bohle RM, et al. Immunhistochemical analysis for expression of calpain 1, calpain 2 and calpastatin in ovarian cancer. Eur J Gynaecol Oncol. 2011;32(6):628-35.
7. Zhang S, Deen S, Storr SJ, Chondrou PS, Nicholls H, Yao A, et al. Calpain system protein expression and activity in ovarian cancer. J Cancer Res Clin Oncol. 2019;145(2):345-61.
8. Shapovalov I, Harper D, Greer PA. Calpain as a therapeutic target in cancer. Expert Opin Ther Targets. 2022;26(3):217-31.
9. Buckley BJ, Whorton AR. Tunicamycin increases intracellular calcium levels in bovine aortic endothelial cells. Am J Physiol. 1997;273(4):C1298-305.
10. Guha P, Kaptan E, Gade P, Kalvakolanu DV, Ahmed H. Tunicamycin induced endoplasmic reticulum stress promotes apoptosis of prostate cancer cells by activating mTORC1. Oncotarget. 2017;15;8(40):68191-207.
11. Kratochvílová K, Horak P, Ešner M, Souček K, Pils D, Anees M, et al. Tumor suppressor candidate 3 (TUSC3) prevents the epithelial-to-mesenchymal transition and inhibits tumor growth by modulating the endoplasmic reticulum stress response in ovarian cancer cells. Int J Cancer. 2015;15;137(6):1330-40.
12. Fang J, Liu X, Bolanos L, Barker B, Rigolino C, Cortelezzi A, et al. A calcium- and calpain-dependent pathway determines the response to lenalidomide in myelodysplastic syndromes. Nat Med. 2016;22(7):727-34.
13. Ono Y, Saido TC, Sorimachi H. Calpain research for drug discovery: challenges and potential. Nat Rev Drug Discov. 2016;15(12):854-76.
14. Chen B, Tang J, Guo YS, Li Y, Chen ZN, Jiang JL. Calpains are required for invasive and metastatic potentials of human HCC cells. Cell Biol Int. 2013;37(7):643-52.
15. Li CL, Yang D, Cao X, Wang F, Hong DY, Wang J, Shen XC, Chen Y. Fibronectin induces epithelial-mesenchymal transition in human breast cancer MCF-7 cells via activation of calpain. Oncol Lett. 2017;13(5):3889-95.
16. Chen Q, Thompson J, Hu Y, Lesnefsky EJ. Tunicamycin-Induced Endoplasmic Reticulum Stress Damages Complex I in Cardiac Mitochondria. Life (Basel). 2022;12(8):1209.
17. Hou H, Ge C, Sun H, Li H, Li J, Tian H. Tunicamycin inhibits cell proliferation and migration in hepatocellular carcinoma through suppression of CD44s and the ERK1/2 pathway. Cancer Sci. 2018;109(4):1088-100.
18. Rose AH, Huang Z, Mafnas C, Hara JH, Hoffmann FW, Hashimoto AS, et al. Calpain-2 Inhibitor Therapy Reduces Murine Colitis and Colitis- associated Cancer Inflamm Bowel Dis. 2015;21(9):2005-15.
19. Lu X, Li G, Liu S, Wang H, Chen B. MiR-585-3p suppresses tumor proliferation and Migration by directly targeting CAPN9 in high grade serous ovarian cancer. J Ovarian Res. 2021;14,90.

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