Cumhuriyet Medical Journal, 44(3): 258-260, 2022
DOI: http://dx.doi.org/10.7197/cmj.1173780

Cumhuriyet Medical Journal

| cmj.cumhuriyet.edu.tr | Founded: 2004 Available online, ISSN:1305-0028 Publisher: Sivas Cumhuriyet Üniversitesi

Anticancer Activity of Calcium Channel Blockers in Colon Cancer Cell Culture

Celal Alandağ[1] *, Ayşegül Öztürk[2]

[1]*Department of Medical Oncology, Sivas Numune Hospital, Sivas, Turkey*

[2]*Vocational School of Health Services, Department of Medical Services and Techniques, Sivas Cumhuriyet University, Sivas, Turkey*

**Corresponding author*

Research Article

History

Received: 11/09/2022
Accepted: 26/09/2022

ABSTRACT

Objective: Repurposing non-cancer drugs for cancer treatment has many advantages. We can access to a new cancer drug easily, quickly and cheaply. In addition, we generally know safety prifile of repurped drugs. There are few studies in the literature investige the anticancer effects of Calcium channel blockers (CCBs). We planned to investigate the anticancer effects of CCBs on colon cancer cell line.

Material and Method: We adminestered different doses of T-type CCB NNC-55-0396 and L-type CCB amlodipine on colon cancer cell line HT-29. MTT analysis was performed at 48 hours to measure cell viability. The dose-response curve was constructed using GraphPad Prism.8 programme.

Results: Amlodipine caused more than 90% cytotoxicity at all concentrations of 500, 250, 100, 50, 10 µg/ml in MTT analysis at 48 hours. Similarly, NNC-55-0396 caused more than 90% cytotoxicity at all 80, 40, 20, 10.5 µM concentrations.

Discussion: In our study, NNC-55-0396 and amlodipine molecules showed severe cytotoxicity on HT-29 cells. There are publications indicating that it may have other anticancer effects other than cytotoxicity. They are promising molecules as anti-cancer drugs. They should be investigated in clinical studies alone or in combination with other cancer drugs.

Keywords: Colon cancer, cell line, amlodipine, calcium chnnel blockers.

Kolon Kanseri Hücre Kültüründe Kalsiyum Kanal Blokerlerinin Antikanser Aktivitesi

Süreç

Geliş: 11/09/2022
Kabul: 26/09/2022

ÖZ

Amaç: Kanser tedavisi için kanser dışı ilaçların yeniden kullanılmasının "repuposing" birçok avantajı vardır. Bu sayede yeni bir kanser ilacına kolay, hızlı ve ucuza ulaşabiliriz. Genel olarak yeniden kullanılan ilaçların güvenlik profilini biliyoruz. Literatürde az sayıda çalışma kalsiyum kanal blokerlerinin (KKB'ler) antikanser etkilerini araştırmıştır. Biz bu çalışmada KKB'lerin kolon kanseri hücre hattı üzerindeki antikanser etkilerini araştırmayı planladık.

Gereç ve Yöntem: Kolon kanseri hücre hattı HT-29'a farklı dozlarda T-tipi KKB (NNC-55-0396) ve L-tipi KKB (amlodipin) uyguladık. Hücre canlılığını ölçmek için 48 saatte MTT analizi yapıldı. Doz-yanıt eğrisi GraphPad Prism.8 programı kullanılarak oluşturulmuştur.

Sonuçlar: Amlodipin, 48 saatte MTT analizinde 500, 250, 100, 50 ve 10 µg/ml'lik tüm konsantrasyonlarda %90'dan fazla sitotoksisiteye neden olmuştur. Benzer şekilde, NNC-55-0396 80, 40, 20 ve 10.5 uM konsantrasyonların hepsinde %90'dan fazla sitotoksisiteye neden olmuştur.

Tartışma: Çalışmamızda NNC-55-0396 ve amlodipin molekülleri HT-29 hücreleri üzerinde ciddi sitotoksisite göstermiştir. Yayınlar, KKB'lerinin sitotoksisite dışında başka antikanser etkileri olabileceğini gösteriyor. Kanser tedavisinde umut vaat eden moleküllerdir. Klinik çalışmalarda tek başlarına veya diğer kanser ilaçları ile birlikte kullanımı araştırılmalıdırlar.

Anahtar sözcükler: Apoptoz, kalsiyum kanal blokeri, hücre hattı, kolon kanseri

[a] *dralandag@hotmail.com* *https://orcid.org/ 0000-0002-2589-8174* | [b] *fztaysegul@yahoo.com* *https://orcid.org/ 0000-0001-8130-7968*

How to Cite: Alandağ C, Öztürk A (2022) Anticancer Activity of Calcium Channel Blockers in Colon Cancer Cell Culture, Cumhuriyet Medical Journal, September 2022, 44 (3): 258-260

Introduction

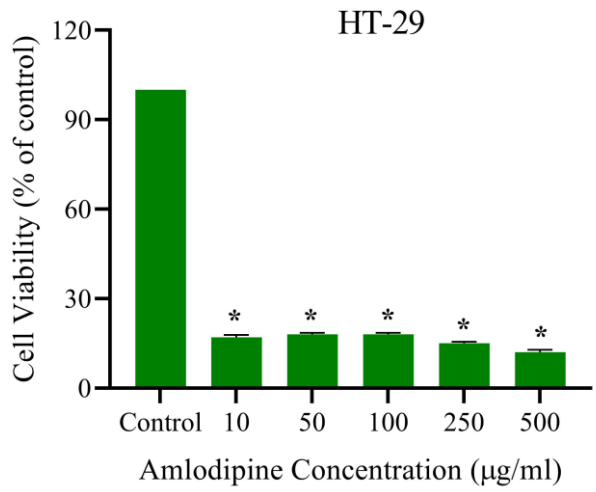
The use of a drug in the treatment of another disease other than its primary indication is called “repurposing” in English. It is very advantageous for a molecule with known safety to get a new indication quickly and with very little cost. A lot of work has been done on the use of molecules that are not cancer drugs before in the treatment of cancer. Among these drugs, many groups such as antihypertensives, diabetes drugs, antibiotics can be counted.[1]. Amlodipine has a special place among repuposed molecules in cancer treatment. In our previous retrospective studies, we have shown that especially regorafenib and amlodipine combination increases the effectiveness at colorectal cancer [2]. In out another study, we showed that amlodipine inproves outcomes of erlotinib in non-small cell lung cancer [3]. In preclinical studies, amlodipine has been shown to inhibit the growth of gastric cancer cell lines.[4]. In addition, the anticancer activity of NNC-55-0396 molecule, which is a T-type calcium channel blocker, has been demonstrated in a colon cancer cell line. In this study, we planned to investigate the cytotoxic effects of amlodipine and NNC-55-0396 molecules in a colon cancer cell culture line to support our retrospective studies.

Materials and Methods

HT-29 was used as colon cancer cell line. The cell line was obtained from the ATCC website. The active ingredient of amlodipine was obtained as a grant from the Nobel Pharmaceutical Company, and the molecule NNC-55-0396 was purchased from the ATCC site. In this study, it was planned to show the cytotoxic effect of amlodipine and NNC-55-0396 molecule on HT-29 cell line. After both molecules were dissolved with 100% DMSO, the concentrations constituting the experimental groups were prepared with the complete medium (the DMSO ratio in the total volume was adjusted as ≤0.1% at this stage). Amlodipine prepared at concentrations of 500, 250, 100, 50, 10 µg/ml and NNC-55-0396 prepared at concentrations of 80, 40, 20, 10.5 µM were added to HT-29 cells. Cell viability was measured by MTT staining of incubated cells at 48th hour. IC50 values were not calculated, IC50 values found in previous similar experiments were used. The dose-response curve was calculated by entering logarithm values to the "nonlinear regression" analysis data of the GraphPad Prism.8 program. The One-way Anova Method was used when comparing the control group (line with DMSO) and the drug-administered groups in terms of cell viability, and a P value of <0.01 was considered significant.

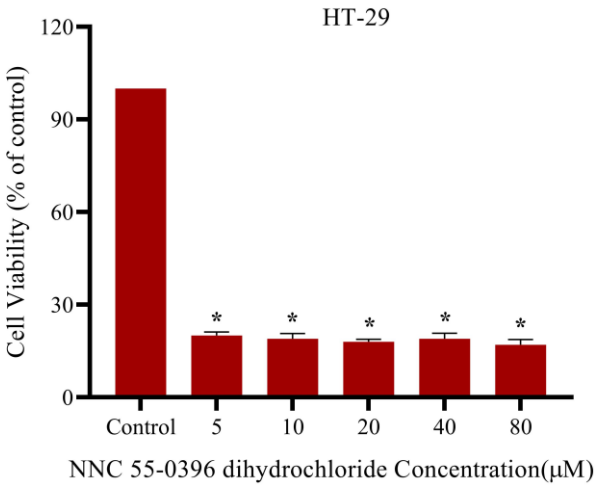
Results

Amlodipine caused more than 90% cytotoxicity at all concentrations of 500, 250, 100, 50, 10 µg/ml in MTT analysis at 48 hours (p<0.01) (Figure 1). Similarly, NNC-55-0396 caused more than 90% cytotoxicity at all 80, 40, 20, 10.5 µM concentrations (p<0.01) (Figure 2).



*p<0.01

Figure 1. The cytotoxic effects of amlodipine at different doses in the HT-29 colon cancer cell line at 48 hours.



*p<0.01

Figure 2. Cytotoxic effects of NNC-55-0396 at different doses in the HT-29 colon cancer cell line at 48 hours.

Discussion

In our study, even at the lowest dose of 10 µg/ml, it showed a serious cytotoxic effect on HT-29 cells. Amlodipine is an L-type voltage-dependent calcium channel blocker. It reduces the amount of calcium in the cell. The amount of calcium in the cell plays an important role in reaching the growth stimuli from the growth receptors to the DNA. Especially fast (peak) and slow changes (oscillation) in the amount of calcium are effective in the transmission of growth stimuli.

Ponmathi et al. [5] showed that the cytotoxic effect of amlodipine on gastric cancer cells was due to inhibition of MAP kinase and TGF-beta pathway. Yoshida et al. [6] showed that epidermal growth factor (EGF)-induced EGF receptor phosphorylation of amlodipine decreased in epidermoid carcinoma cells. In our study, cytotoxicity similar to amlodipine was observed with NNC-55-0396 molecule. NNC-55-0396 is a T-type voltage-dependent calcium channel blocker. It pumps calcium into the cell from both the cell membrane and the endoplasmic reticulum. In its blockade, the amount of cytoplasmic calcium and its oscillation decrease. Huang et al. [7] showed that the NNC-55-0396 molecule has a cytotoxic effect by inhibiting the ERK pathway in a colon cancer cell line. The anti-cancer effects of CCBs have also been explained by other mechanisms. In particular, it has been shown to reduce the level of programmed death ligand-1 in tumor cells. In this way, the immune response against the tumor increases. In addition, it has been stated that it can exert an anti-cancer effect by changing the tumor microenvironment [6, 8].

In our study, it was shown that similar results were obtained with different calcium channel blockers. Obtaining anti-cancer activity is thought to be due to the molecules changing the intracellular calcium amounts rather than blocking the specific targets. CCBs are known to inhibit the peak and oscillation of cytoplasmic amounts of calcium. NNC-55-0396 molecule is not used in clinical practice. Amlodipine is frequently used in the treatment of arterial hypertension. The safety and toxicity profile is well known. It is a candidate molecule for prospective clinical study in cancer patients. For this reason, the anticancer efficacy mechanisms of amlodipine should be clarified in all aspects, and its anticancer properties should be revealed with more preclinical and clinical studies.

References

1. Corsello, S.M., et al., Discovering the anti-cancer potential of non-oncology drugs by systematic viability profiling. Nature cancer, 2020. 1(2): p. 235-248.
2. Alandağ, C., E. Karaman, and E. Yüce, Amlodipine improves the outcomes of regorafenib in metastatic colorectal cancer. Anti-Cancer Drugs, 2022. 33(4): p. 389-393.
3. Alandağ, C., E. Merev, and F. Özdemir, Repurposing calcium channel blockers: may be sensible combination with erlotinib for non-small cell lung cancer. Anticancer Drugs, 2021.
4. Shiozaki, A., et al., Amlodipine and Verapamil, Voltage-Gated Ca(2+) Channel Inhibitors, Suppressed the Growth of Gastric Cancer Stem Cells. Ann Surg Oncol, 2021. 28(9): p. 5400-5411.
5. Panneerpandian, P., D.B. Rao, and K. Ganesan, Calcium channel blockers lercanidipine and amlodipine inhibit YY1/ERK/TGF-β mediated transcription and sensitize the gastric cancer cells to doxorubicin. Toxicol In Vitro, 2021. 74: p. 105152.
6. Yoshida, J., et al., Amlodipine, a Ca2+ channel blocker, suppresses phosphorylation of epidermal growth factor receptor in human epidermoid carcinoma A431 cells. Life Sci, 2010. 86(3-4): p. 124-32.
7. Huang, W., et al., T-type calcium channel antagonists, mibefradil and NNC-55-0396 inhibit cell proliferation and induce cell apoptosis in leukemia cell lines. Journal of Experimental & Clinical Cancer Research, 2015. 34(1): p. 54.
8. Bong, A.H.L. and G.R. Monteith, Calcium signaling and the therapeutic targeting of cancer cells. Biochim Biophys Acta Mol Cell Res, 2018. 1865(11 Pt B): p. 1786-1794.