*Article*

# Anticancer Activity of Selective and Non-Selective Beta Adrenoreceptor Blockers against Non-Small Cell Lung Cancer Cell Lines

**Marina Sidorova [1] and Vilma Petrikaite [1,*]**

[1] Faculty of Pharmacy, Lithuanian University of Health Sciences, A. Mickeviciaus g. 9, LT-44307 Kaunas, Lithuania; vilma.petrikaite@lsmuni.lt

* Correspondence: vilma.petrikaite@lsmuni.lt; Tel.: +37068629383

**Abstract:** Beta adrenoblockers is a large class of drugs mainly used to manage abnormal heart rhythms. Over the last couples of decades, the anticancer effects of these compounds has been extensively studied. There is much evidence about their activity in non-small cell lung, pancreatic, breast, colorectal, prostate and ovarian cancer. However, the mechanism of beta blockers anticancer activity is still not known, and more detailed studies are needed.

The aim of our study was to evaluate the anticancer activity of beta adrenoblockers in non-small cell lung cancer cell lines A549 and H1299. In order to find the relationship with their selectivity to beta adrenoreceptors, in our study we used selective (atenolol, betaxolol, esmolol, metoprolol) and non-selective (pindolol, propranolol and timolol) beta blockers. The effect on cell viability was evaluated by MTT assay and the activity on cell ability to form colonies was tested by clonogenic assay. The type of cell death was evaluated by cell double staining with Hoechst 33342 and Propidium iodide.

The most active adrenoblockers against both tested cancer cell lines were propranolol and betaxolol. They completely inhibited lung cancer cell colony formation at 90% of $EC_{50}$ (half maximal effective concentration) value. Most tested compounds induced cell death through apoptosis and necrosis. In A549 cell lines apoptosis was mainly induced while in H1299 cell line compounds induced both apoptosis and necrosis. There was no correlation established between beta adrenoblocker anticancer activity and their selectivity to beta adrenoreceptors.

**Keywords:** beta adrenoblocker, anticancer, non-small cell lung cancer, clonogenic, apoptosis, necrosis

## 1. Introduction

Lung cancer is the most common type of cancer and a leading cause of death worldwide, accounting for an estimated 9.6 million deaths [1]. Despite progress in diagnostics and treatment, lung cancer therapy remains problematic. Resistance to drugs is the main reason decreasing effectiveness of therapy [2] and 5-year survival rate is less than 18% [3].

Catecholamines norepinephrine and epinephrine, also called noradrenaline and adrenaline, are neurotransmitters simultaneously released from sympathetic nervous system and adrenal gland as a response to physiological and psychological stress, otherwise called flight-of-fight response. They regulate the activity of organs and cells, related to stimulation of sympathetic nerve system. According to scientists, elevated concentration of catecholamines promotes growth of lung adenocarcinoma micro metastasis [1, 7]. Conversely, beta adrenergic receptor antagonists (beta

 doi:10.20944/preprints201907.0279.v1



adrenoblockers) by binding to the beta adrenoreceptors stop binding of norepinephrine and epinephrine at these receptors, thereby decreasing their stimulation and risk of the growth of cancer.

Beta adrenoblockers is a large class of drugs mainly used to manage abnormal heart rhythms. Over the last couples of decades the anticancer effects of these compounds has been extensively studied.The first evidence about beta adrenoreceptor involvement in lung cancer development occurred in 1989 [4]. According to recent studies, beta blockers also possess anticancer activity in pancreatic, breast, colorectal, prostate and ovarian cancer [2,3,5]. The researchers concluded that stimulation of beta adrenoreceptors by catecholamines leads to increase of extracellular concentration of cyclic adenosine monophosphate, which promotes proliferation of cancer cells [6]. Catecholamines are also involved in immune system functioning. These neuromediators promote resistance of cancer cells and tumor formation and growth by decreasing the amount and activity of lymphocytes and natural killer cells [7]. In animal models, antagonistic effect of non-selective beta adrenoreceptors blockers on beta-2 adrenoreceptors reactivated functioning of lymphocytes but did not improve survival outcomes [8]. However, beta adrenoblocker in combination with COX-2 inhibitors improved survival rates of mice [9]. It was proven that through the activation of beta adrenoreceptors COX-2 becomes active and therefore cancer cell growth and invasion is promoted through arachidonic acid pathway [12, 13].

In preclinical studies, beta blockers have been shown to reduce the proliferation, migration, invasiveness, angiogenesis of cancer cells and tumor immune response [8,12,13]. The exact antitumor mechanism of action of this class of drugs remains unclear and it is therefore important to carry out more detailed studies in cancer cell lines.

According to the results of clinical trials, beta adrenoblockers increase the survival rate of patients suffering from breast, prostate, ovarian, colorectal, skin and lung cancer. In recent years, new evidence has shown that overall survival of patients, who received beta adrenoblockers combined with radiotherapy, increased by 22% comparing to the control group [14]. Despite the evidence of positive effect of beta adrenoblockers on patients' survival some data shows that intake of beta adrenoblockers and other medicines, affecting metabolism of catecholamines, is associated with increased risk of development of cancer and higher mortality [16, 17].

Although anticancer activity of beta adrenoblockers has been observed for almost two decades, there are not enough studies done to clarify their activity in non-small cell lung cancer (NSCLC) cell lines. Considering the problem and prevalence of lung cancer treatment, we decided to investigate the anticancer activity of beta adrenoblockers in NSCLC lines A549 and H1299. In this work, their effect on cell viability, clonogenicity and the type of cell death was investigated. Also, for studies we used bot selective and non-selective beta blockers to explore possible relationship between their anticancer activity and selectivity to beta adrenoreceptors.

## 2. Results

### Beta adrenoblockers reduce the viability of NSCLC cells

All tested compounds reduced NSCLC cell viability at the highest used concentration of 500 μM (Figure 1a). Propranolol and betaxolol were the most active compounds in both cell lines. Propranolol showed a stronger effect on viability of H1299 cell line, while betaxolol acted in the same way in both cell lines ($p < 0.05$) (Figure 1b).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0279.v1



Propranolol possessed the highest antiproliferative activity ($EC_{50}$ values were 119.3 ± 12.7 μM and 98.8 ± 10.3 μM in A549 and H1299 cell lines, respectively). Betaxolol activity was about twice lower compared to propranolol ($EC_{50}$ values were 251.3 ± 14.6 μM and 252.2 ± 7.6 μM in A549 and H1299 cell lines, respectively).

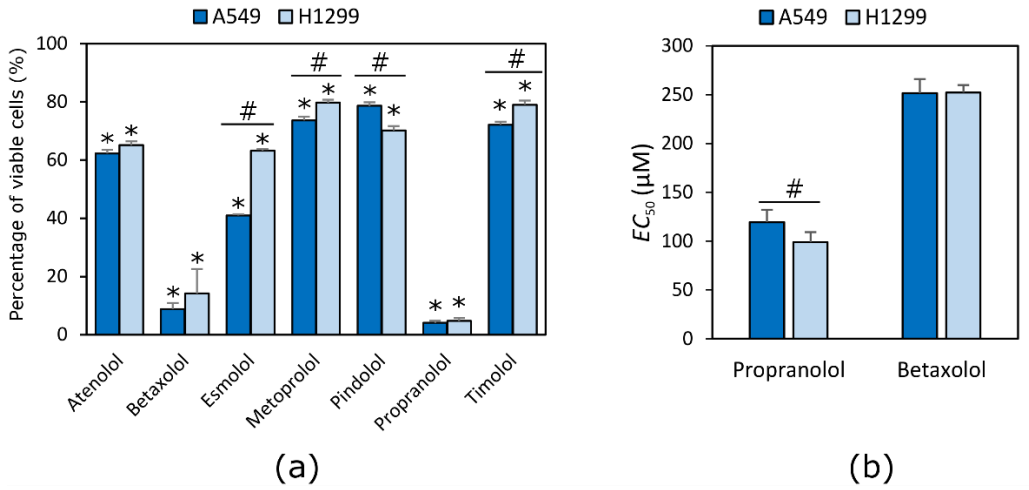


**Figure 1.** Effect of beta adrenoblockers on NCLSC cell viability. (a) effect of all tested compounds 500 μM concentration on A549 and H1299 cell viability; (b) $EC_{50}$ values of propranolol and betaxolol. * $p < 0.05$, compared to control; # $p < 0.05$, compared activity between cancer cell lines.

**Beta adrenoblockers inhibit growth of cell colonies in concentration-dependent way**

Tested beta adrenoblockers showed different effect on NCLSC cell colony formation (Figure 2). Propranolol and betaxolol at a concentration of 90% of $EC_{50}$ value completely suppressed colony formation ability in both cell lines ($p < 0.05$) (Figure 3a and 3b). All compounds except for atenolol at the higher concentration inhibited growth of cells colonies.

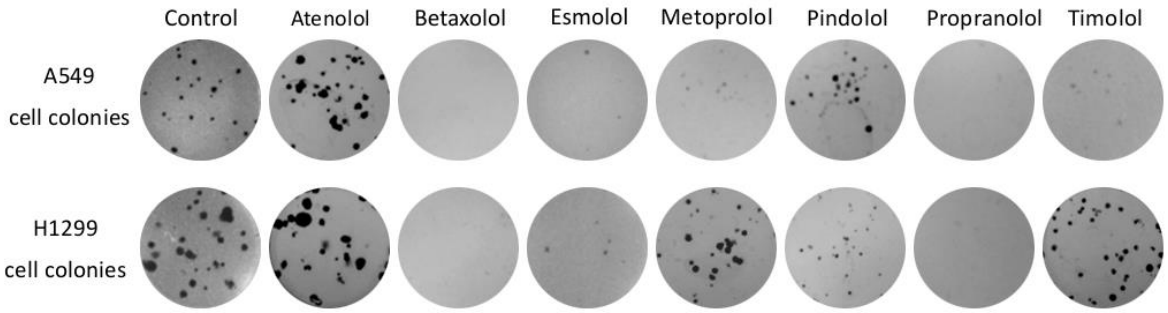


**Figure 2.** A549 and H1299 cell colonies after incubation with 90% of $EC_{50}$ concentrations of beta adrenoblockers.

Slightly weaker than propranolol and betaxolol, the number and area of A549 cell colonies was reduced by metoprolol at the higher used concentration in this study. Non-selective beta blockers timolol and pindolol and selective beta blocker esmolol were found to possess lower activity compared to propranolol and betaxolol, but the similar one between them. The lowest A549 cell colony formation ability was established for selective beta blocker atenolol.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0279.v1



Similar trends have been identified in the study of the effect of beta blockers on H1299 cell line. All compounds except atenolol had a statistically significant reduction in the number of colonies and area occupied by these cells ($p < 0.05$). The most active were esmolol and metoprolol ($p < 0.05$).

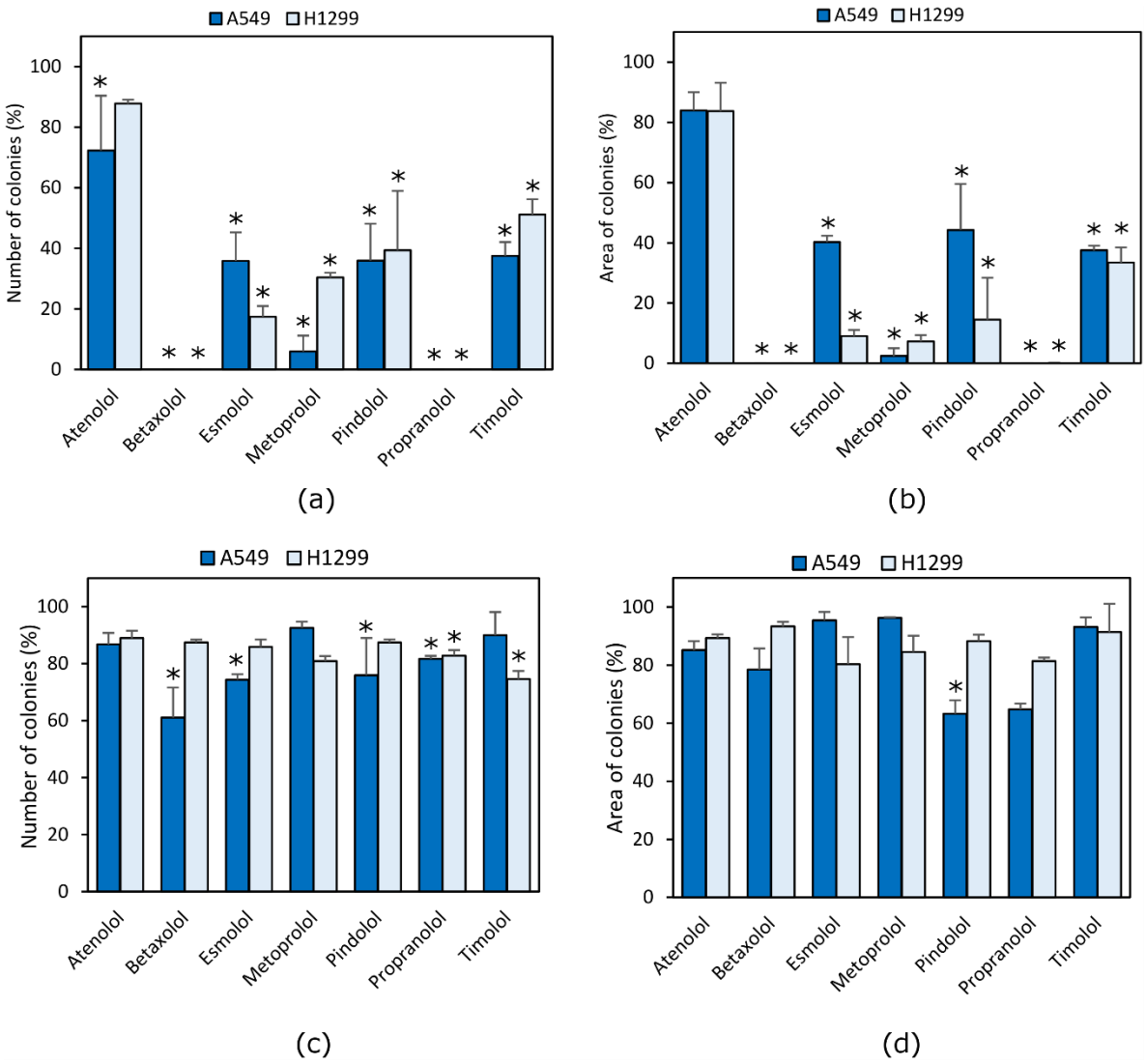


**Figure 3.** Effect of beta adrenoblockers on NCLSC cell colony formation ability. Comparison of compound effect of 90% of their $EC_{50}$ value on (a) cell colony number and (b) area of colonies; and compound effect of 10% of EC50 value on (c) cell colony number and (d) area of colonies. * $p < 0.05$, compared to control.

Only betaxolol at a concentration of 10% of $EC_{50}$ value inhibited growth of A549 cells colonies, while pindolol and propranolol also decreased a size of colonies, comparing to the control group ($p < 0.05$) (Figure 3c and 3d). None of the compounds at lower concentration had effect on growth and size of H1299 cells colonies ($p > 0.05$).

**Beta adrenoblockers mainly cause apoptosis**

Most tested compounds induced cell death through apoptosis and necrosis. In A549 cell lines apoptosis was mainly induced while in H1299 cell line compounds induced both apoptosis and necrosis (Figure 4).

All the tested compounds induced apoptosis in A549 cell line even at concentration of 10% of calculated $EC_{50}$ value ($p < 0.05$). No statistically significant difference was found between apoptotic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0279.v1



effect of beta adrenoblockers at concentration of 10 and 90% of calculated $EC_{50}$ value on A549 cells ($p > 0.05$). Only atenolol at both concentrations and betaxolol at lower concentration did not induce apoptosis in H1299 cell line ($p > 0.05$).

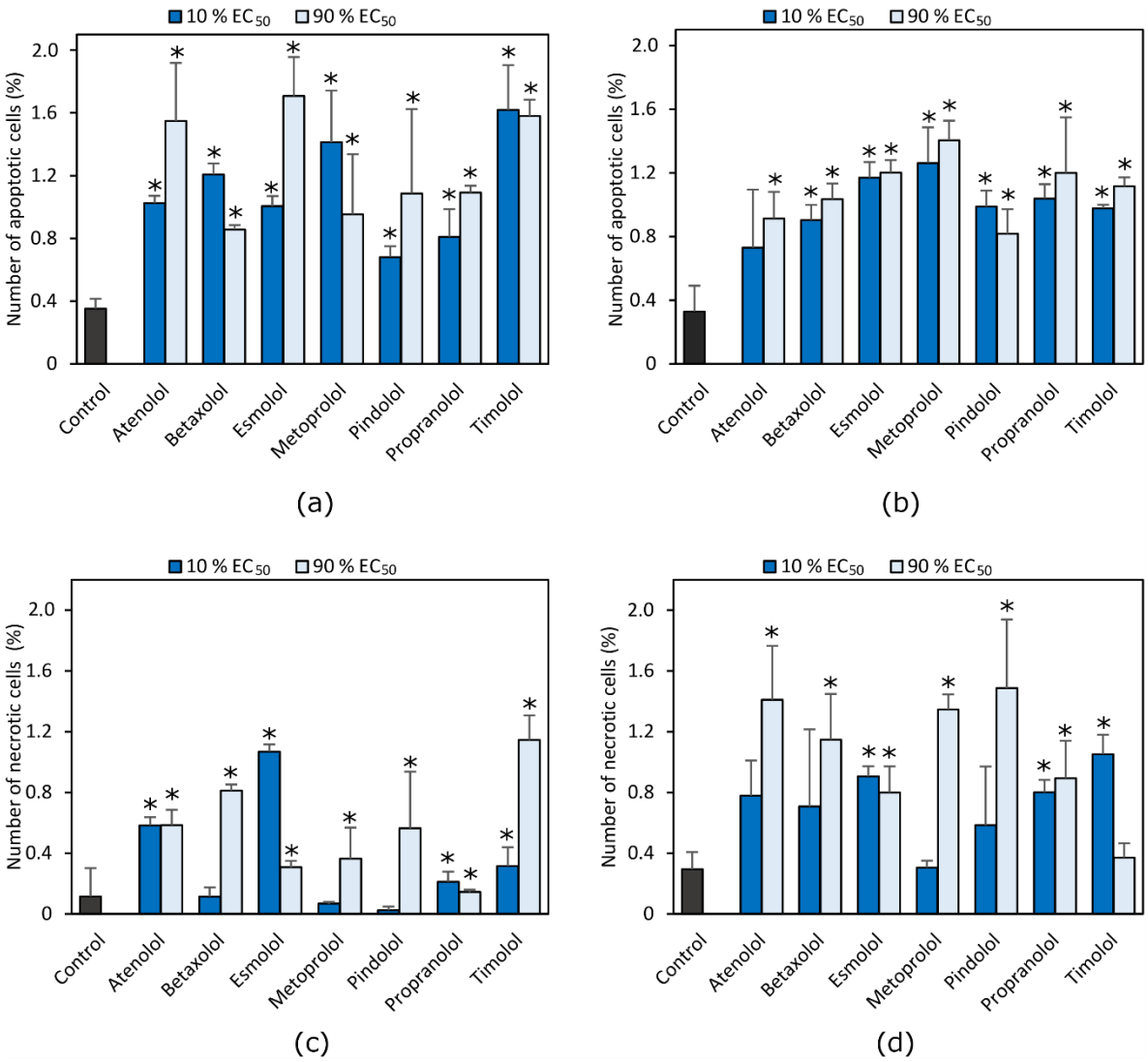


**Figure 4. Effect of beta adrenoblockers on NCLSC cell death type. Number of apoptotic cells in** (**a**) A549; (**b**) H1299 cancer cell lines; and number of necrotic cells in (**c**) A549 and (**d**) H1299 cell lines. * $p < 0.05$, compared to the control

No statistically significant difference was found between beta adrenoblockers effect on cell apoptosis in both cell lines.

Beta adrenoblockers mainly induced necrosis in H1299, but not in A549 cell line. All of the tested compounds with the exception of timolol induced necrosis in H1299 cell line ($p < 0.05$). Metoprolol, pindolol and propranolol almost did not cause necrosis of A549 cells and at lower concentrations did not have effect on H1299 cells ($p > 0.05$).

## 3. Discussion

Effect of beta adrenoblockers on cell viability is common subject of different scientific studies. Propranolol after 72 h of incubation inhibited cell viability of lung cancer A375 and melanoma P8 cell lines at concentrations 77.30 and 60.30 μM, respectively [17]. Similar results were obtained in

 doi:10.20944/preprints201907.0279.v1



myeloma U266 cell line [18]. Difference between calculated $EC_{50}$ values can be explained by the fact that expression of receptors varies between different types of cell lines. In general, it is thought that non-selective beta adrenoblockers possess stronger effect on cell viability than beta-1 selective compounds [19]. Atenolol was from 7 to 50 times less active than propranolol in breast MCF-7, colorectal HT-29 and hepatocellular HepG2 cell lines. Similar results were achieved in this study. Atenolol was 6 times less active than propranolol. The amount of living cells after exposure to atenolol was 62.26 % in H1299 and 65.12 % in A549 cell line, comparing to 4.13% and 4.73%, comparing to a propranolol. Propranolol is non-selective beta adrenoblocker whilst atenolol is selective. Moreover, propranolol possesses membrane stabilizing activity. However, this tendency was not noticed in examining activity of the rest five substances used in the experiment. One of the most active compounds – betaxolol – is selective and propranolol is non-selective beta adrenoblocker.

We found that antiproliferative activity of beta adrenoblockers is not correlating with their selectivity to the receptors and might be dependent on the compound lipophilicity and membrane stabilizing activity. Beta-2 adrenoreceptors in lung adenocarcinoma are responsible for lymphatic permeation and vascular invasion [20]. However, beta-2 adrenoreceptor expression in lung adenocarcinoma is not associated with worse survival outcomes in patients. In this study, only one of the non-selective beta adrenoblockers, propranolol, inhibited cell viability at concentration less than 500 μM. Betaxolol and propranolol possess the same selectivity to beta-1 adrenoceptors. However non-selective pindolol with the strongest beta-1 antagonistic activity of all the tested compounds was the least active compound in A549 cell line, but one of the most active compounds in H1299 cell line. Selective adrenoblockers esmolol and atenolol also were one of the most active compounds in H1299 cells, what might be a proof that expression of adrenoreceptors varies in cell lines themselves and selectivity of compounds is not the most important feature in order to predict anticancer activity of a substance. Zhang and the group suggested that activated k-ras gene mutation in cell lines might be responsible for the lower activity of beta-2 adrenoreceptor blockers [21]. This explains why propranolol was more active in H1299 cell line ($p < 0.05$), while betaxolol activity was the same in both cell lines ($p > 0.05$).

Effect of beta adrenoblockers on colony formation is not a common subject of scientific researches. Min and the group discovered that propranolol and atenolol at 10 μM concentrations suppress the growth and ability of A549 and H0CC-15 cells, treated by NNK, to form colonies [22]. In this study, 12 μM concentration propranolol reduced the size of A549 cell colonies, but atenolol even at 450 μM concentration did not have a statistically significant effect on colony growth. The deviation from expected results can be explained by the difference between cell lines and also methods of cultivation used in research. There is also evidence that propranolol in combination with radiotherapy and sumatinib reduces clonogenicity of stomach cancer and melanoma [17, 23].

Zhang and the group concluded that 100 μM concentration metoprolol does not cause apoptosis in pancreatic cell lines [21]. In this study metoprolol even at 50 μM concentration induced apoptosis in A549 and H1299 cell lines. The results of experiments may differ due to variation of expression of beta adrenoreceptors in cell lines and mechanism of action of drugs through metabolic pathways.

In another study propranolol at 50 μM concentration did not cause apoptosis of gastric adenocarcinoma BGC-823 and SGC-7901cell lines, but in combination with radiotherapy after 48 h incubation it induced apoptosis, clonogenic survivability and cell viability [23]. In our experiment

 doi:10.20944/preprints201907.0279.v1



propranolol induced apoptosis at 12 μM concentration, however, cells were incubated with solutions of compounds 72 h. Ability of beta adrenoblockers to cause apoptosis may be time-dependent.

In order to evaluate impact of beta adrenergic receptors on type of cell death, the effect of beta-2 selective adrenoblocker butoxamine, non-selective propranolol and beta-1 selective metoprolol were used to induce apoptosis in PC-2 pancreatic cancer cell line [24]. The apoptosis rate was the lowest after treatment with metoprolol, the highest – after treatment with butoxamine. According to the results of this study, it can be stated that apoptotic effect of beta adrenoblockers is mainly dependent on selectivity to beta-2 adrenoreceptors. It is worth noting that Zhang and others used only single compounds that possess specific selectivity to a certain type of receptors. In our study, lung cancer cell lines were treated with several different compounds possessing different selectivity towards beta adrenoreceptors, but no statistically significant differences between their effect were noticed. However, both NSCLC cell lines, A549 and H1299, possess K-Ras gene mutation that is thought to be responsible for lower sensitivity to non-selective beta adrenoblockers [21]. Moreover, different concentrations of compounds were used. It may be presumed that selectivity of beta adrenoblockers is important for anticancer activity in some specific cell lines, but not all of them in general.

## 4. Materials and Methods

### *4.1. Chemicals and materials*

Atenolol (99% pure), betaxolol (96% pure), esmolol (98% pure), timolol (99% pure) and pindolol (99% pure) were purchased from Abcam (Cambridge, UK), metoprolol (98% pure) from Alfa Aesar (Massachusetts, USA) and propranolol (99% pure) from Acros Organic (New Jersey, USA). All tested compounds were dissolved in dimethylsulfoxide (DMSO, ≥99%, Ph. Eur.) which was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

TrypLE Express, Dulbecco's modified Eagle high glucose medium (DMEM GlutaMAX), fetal bovine serum (FBS), penicillin/streptomycin solution (10,000 IU/mL), phosphate buffered saline (PBS, pH 7.4) were purchased from (Gibco, Carlsbad, CA, USA). Aqueous 16% paraformaldehyde solution (PFA), Hoechst 33342 (1 mg/mL) solution and Propidium iodide (1 mg/mL) solution were obtained from Thermo Fisher Scientific, UK.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, ≥ 97%) and crystal violet (≥90%) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Ethanol (96.6%) was obtained from Stumbras, LLC (Kaunas, Lithuania).

All cell culture plastic ware was purchased from Thermo Fisher Scientific, Corning and Techno Plastic Products.

### *4.2. Cell culture*

Human NSCLC cell lines A549 and H1299 were obtained from prof. Esteller Manel (The Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain). Both cell lines were cultured in Dulbecco's Modified Eagle's Medium GlutaMAX, supplemented with 10% FBS and 1% antibiotics. Cells were incubated at 37°C temperature in a humidified atmosphere containing 5% $CO_2$. All cell cultures routinely were grown to 70% confluence and trypsinized with 0.125% TrypLE™ Express solution before passage. They were used until passage 20.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0279.v1



*4.3. Cell viability assay*

Cell viability was evaluated by MTT assay. A549 and H1299 cells were seeded in a 96-well plate at a concentration 5,000 cells/well and incubated overnight. After 24 h, cells were affected by different concentrations of beta adrenoblockers. Medium without cells served as a positive control, and the cells treated with medium containing 0.5% DMSO was used as a negative control.

After 72 h, 20 µL of MTT 0.5 mg/mL solution was added into each well of a 96-well plate and cells were incubated at 37°C for 3 hours. Then supernatant was removed and formed formazan crystals were dissolved in 100 µL of DMSO. The absorbance was measured at 570 nm and 630 nm reference wavelengths using a multi-detection microplate reader. Experiments were repeated three times independently and the results were presented as means ± SD.

Applying Hill fit to compound dose – cell metabolic activity (absorbance) curves, the half maximal effective concentration ($EC_{50}$) values, reducing cell viability by 50%, were calculated.

*4.4. Cell colony formation assay*

1000 of A549 and H1299 cells in a volume of 1 ml were seeded in 12-well and then were treated with 100 µL of 10 or 90% of $EC_{50}$ values of adrenoblockers. Medium containing 0.5% of DMSO served as a negative control. H1299 cells were incubated for 8 days, and A549 for 12 days at 37°C in an atmosphere containing 5% $CO_2$. Then the colonies were rinsed twice with PBS and fixed with 4% paraformaldehyde solution in PBS for 15 min. Colonies were stained with 0.1% aqueous crystal violet solution for 15 min and washed twice with sterile deionized water. Pictures were taken using G:BOX gel documentation system (Syngene International Ltd, Bengaluru, India) and analysed using Genesys software (Syngene International Ltd). The number and percentage area of colonies were calculated.

*4.5. Evaluation of type of cell death*

Lung cancer cells were seeded in 24-well plates at a concentration 15,000 cells/well and incubated for 24 h at 37°C in an atmosphere containing 5% $CO_2$. Then 10 or 90% of $EC_{50}$ values of adrenoblockers were added to the wells. After 72 h 3 µL of aqueous solution of Hoechst 33342 (1 mg/mL) and 1 µL of aqueous solution of Propidium iodide (1 mg/mL) were added to each well. After 10 min, images of cells were taken by inverted fluorescent microscope (Olympus IX73). Apoptotic and necrotic cells were counted, and the percentage number of cells was calculated.

*4.6. Statistical analysis*

Statistical analysis was performed using *Microsoft Office Excel 2007* software (Microsoft Corporation, Redmond, WA, USA), evaluating an average and standard deviation of, at least, 3 measurements. Student's t-test was used, and p-values were calculated. A value of $p < 0.05$ was considered as the level of significance.

## 5. Conclusions

Our results show that both selective and non-selective beta adrenoblockers, especially betaxolol and propranolol, reduce the viability of NSCLC cell lines H1299 and A549. Betablockers inhibit formation of cell colonies and induce apoptosis and necrosis. Anticancer activity of tested beta adrenoblockers is not related to the selectivity to beta adrenoreceptors.

**Author Contributions:** M.S. performed the experiments, analysed the data and wrote the manuscript. V.P. designed the experiments, was involved in analysis of data and revised the paper.

**Funding:** This research received no external funding.

**Acknowledgments:** In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. - PubMed - NCBI [Internet]. [cited 9 Jul 2019]. Available: https://www.ncbi.nlm.nih.gov/pubmed/30207593

2. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013;13: 714–726. doi:10.1038/nrc3599

3. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64: 9–29. doi:10.3322/caac.21208

4. Schuller HM, Cole B. Regulation of cell proliferation by beta-adrenergic receptors in a human lung adenocarcinoma cell line. Carcinogenesis. 1989;10: 1753–1755.

5. Park PG, Merryman J, Orloff M, Schuller HM. Beta-adrenergic mitogenic signal transduction in peripheral lung adenocarcinoma: implications for individuals with preexisting chronic lung disease. Cancer Res. 1995;55: 3504–3508.

6. Bernabé DG, Tamae AC, Biasoli ÉR, Oliveira SHP. Stress hormones increase cell proliferation and regulates interleukin-6 secretion in human oral squamous cell carcinoma cells. Brain Behav Immun. 2011;25: 574–583. doi:10.1016/j.bbi.2010.12.012

7. Shakhar G, Ben-Eliyahu S. In Vivo β-Adrenergic Stimulation Suppresses Natural Killer Activity and Compromises Resistance to Tumor Metastasis in Rats. J Immunol. 1998;160: 3251–3258.

8. Cole SW, Sood AK. Molecular pathways: beta-adrenergic signaling in cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2012;18: 1201–1206. doi:10.1158/1078-0432.CCR-11-0641

9. Glasner A, Avraham R, Rosenne E, Benish M, Zmora O, Shemer S, et al. Improving survival rates in two models of spontaneous postoperative metastasis in mice by combined administration of a beta-adrenergic antagonist and a cyclooxygenase-2 inhibitor. J Immunol Baltim Md 1950. 2010;184: 2449–2457. doi:10.4049/jimmunol.0903301

10. Wong HPS, Yu L, Lam EKY, Tai EKK, Wu WKK, Cho C-H. Nicotine promotes colon tumor growth and angiogenesis through beta-adrenergic activation. Toxicol Sci Off J Soc Toxicol. 2007;97: 279–287. doi:10.1093/toxsci/kfm060

11. Wong HPS, Yu L, Lam EKY, Tai EKK, Wu WKK, Cho CH. Nicotine promotes cell proliferation via alpha7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated pathway in human colon adenocarcinoma HT-29 cells. Toxicol Appl Pharmacol. 2007;221: 261–267. doi:10.1016/j.taap.2007.04.002

12. Goldfarb Y, Sorski L, Benish M, Levi B, Melamed R, Ben-Eliyahu S. Improving postoperative immune status and resistance to cancer metastasis: a combined perioperative approach of immunostimulation and prevention of excessive surgical stress responses. Ann Surg. 2011;253: 798–810. doi:10.1097/SLA.0b013e318211d7b5

13. Yang EV, Kim S, Donovan EL, Chen M, Gross AC, Webster Marketon JI, et al. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun. 2009;23: 267–275. doi:10.1016/j.bbi.2008.10.005

14. Wang HM, Liao ZX, Komaki R, Welsh JW, O'Reilly MS, Chang JY, et al. Improved survival outcomes with the incidental use of beta-blockers among patients with non-small-cell lung cancer treated with definitive radiation therapy. Ann Oncol Off J Eur Soc Med Oncol. 2013;24: 1312–1319. doi:10.1093/annonc/mds616

15. Hajighasemi F, Mirshafiey A. In Vitro Sensitivity of Leukemia Cells to Propranolol. J Clin Med Res. 2009;0: 144–149. doi:10.4021//jocmr.v0i3.81

16. Lee J-W, Shahzad MMK, Lin YG, Armaiz-Pena G, Mangala LS, Han H-D, et al. Surgical stress promotes tumor growth in ovarian carcinoma. Clin Cancer Res Off J Am Assoc Cancer Res. 2009;15: 2695–2702. doi:10.1158/1078-0432.CCR-08-2966

17. Kuang X, Qi M, Peng C, Zhou C, Su J, Zeng W, et al. Propranolol enhanced the anti-tumor effect of sunitinib by inhibiting proliferation and inducing G0/G1/S phase arrest in malignant melanoma. Oncotarget. 2018;9: 802–811. doi:10.18632/oncotarget.22696

18. Kozanoglu I, Yandim MK, Cincin ZB, Ozdogu H, Cakmakoglu B, Baran Y. New indication for therapeutic potential of an old well-known drug (propranolol) for multiple myeloma. J Cancer Res Clin Oncol. 2013;139: 327–335. doi:10.1007/s00432-012-1331-y

19. İşeri ÖD, Sahin FI, Terzi YK, Yurtcu E, Erdem SR, Sarialioglu F. beta-Adrenoreceptor antagonists reduce cancer cell proliferation, invasion, and migration. Pharm Biol. 2014;52: 1374–1381. doi:10.3109/13880209.2014.892513

20. Yazawa T, Kaira K, Shimizu K, Shimizu A, Mori K, Nagashima T, et al. Prognostic significance of β2-adrenergic receptor expression in non-small cell lung cancer. Am J Transl Res. 2016;8: 5059–5070.

21. Zhang D, Ma Q, Wang Z, Zhang M, Guo K, Wang F, et al. β2-adrenoceptor blockage induces G1/S phase arrest and apoptosis in pancreatic cancer cells via Ras/Akt/NFκB pathway. Mol Cancer. 2011;10: 146. doi:10.1186/1476-4598-10-146

22. Min H-Y, Boo H-J, Lee HJ, Jang H-J, Yun HJ, Hwang SJ, et al. Smoking-associated lung cancer prevention by blockade of the beta-adrenergic receptor-mediated insulin-like growth factor receptor activation. Oncotarget. 2016;7: 70936–70947. doi:10.18632/oncotarget.12342

23. Liao X, Che X, Zhao W, Zhang D, Long H, Chaudhary P, et al. Effects of propranolol in combination with radiation on apoptosis and survival of gastric cancer cells in vitro. Radiat Oncol Lond Engl. 2010;5: 98. doi:10.1186/1748-717X-5-98

24. Zhang D, Ma Q, Shen S, Hu H. Inhibition of pancreatic cancer cell proliferation by propranolol occurs through apoptosis induction: the study of beta-adrenoceptor antagonist's anticancer effect in pancreatic cancer cell. Pancreas. 2009;38: 94–100. doi:10.1097/MPA.0b013e318184f50c

**Sample Availability:** Compounds tested in this research are commercially available.