Arch Biol Sci. 2021;73(1):31-38 https://doi.org/10.2298/ABS201010055T

Synergistic effect of 17-allylamino-17-demethoxygeldanamycin with dehydroxymethylepoxyquinomicin on the human anaplastic thyroid carcinoma cell line KTC2

Lidija Todorović[1,*], Gorana Stamenković[2], Biljana Vučetić Tadić[3], Kazuo Umezawa[4,5], Ana Božović[1], Shunichi Yamashita[4,6] and Boban Stanojević[1,4]

[1]*Laboratory for Radiobiology and Molecular Genetics, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade*, Belgrade, Serbia

[2]*Department of Genetic Research, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade*, Belgrade, Serbia

[3]*Institute for Mother and Child Healthcare of Serbia*, Belgrade, Serbia

[4]*Department of Molecular Medicine, Nagasaki University Graduate School of Biomedical Sciences*, Nagasaki, Japan

[5]*Department of Molecular Target Medicine, Aichi Medical University*, Nagakute, Japan

[6]*Fukushima Medical University*, Fukushima, Japan

***Corresponding author**: lidijat@vinca.rs

Received: October 10, 2020; **Revised**: December 17, 2020; **Accepted**: December 18, 2020; **Published online**: December 25, 2020

Abstract: The use of targeted inhibitors has shown promise as an effective approach in cancer therapy. However, targeted therapies based only on one drug, such as 17-allylamino-17-demethoxygeldanamycin (17-AAG), have limited success, partly because cancer cells engage alternate pathways for survival and proliferation. In the present study, we evaluated whether dehydroxymethylepoxyquinomicin (DHMEQ), a nuclear factor κB (NF-κB) inhibitor, can enhance the antitumor activities of 17-AAG, a 90 kDa heat shock protein (Hsp90) inhibitor, in the anaplastic thyroid cancer cell line KTC2. We examined the effect of combined drug treatment vs single drug treatment on cell survival. Isobologram analysis was performed to distinguish the additive vs synergistic effects of the drug combination. Western blotting was performed to investigate apoptosis markers: caspase 3, poly(ADP-ribose) polymerase-one (PARP-1), B-cell lymphoma-extra large (Bcl-XL), X-linked inhibitor of apoptosis (XIAP) and cellular inhibitor of apoptosis 2 (cIAP-2). Compared to monotherapy, the combined treatment enhanced growth-inhibitory effects in a synergistic manner and strongly potentiated apoptosis. These results demonstrate the first *in vitro* evidence that a combination of Hsp90 and NF-κB inhibitors is a more effective modality for inhibiting cell proliferation and survival in anaplastic thyroid carcinoma cells than either agent alone, warranting further investigations.

Keywords: NF-κB inhibitor; Hsp90 inhibitor; synergy; targeted inhibitor; combined treatment

INTRODUCTION

Anaplastic thyroid cancer (ATC) is a rare, undifferentiated thyroid cancer, with a high malignant potential and poor prognosis. The five-year survival rate from this type of cancer is less than 5%, with most patients dying within 3 to 6 months of diagnosis despite conventional multimodality treatment strategies, which include surgery, external beam radiotherapy and chemotherapy [1,2]. Treatment failure in ATC is often associated with resistance to chemotherapy drugs. Thus, there is a pressing need for more effective therapeutic approaches, such as those including targeted inhibitors, specially when used in combination. Targeted inhibitors act on specific target molecules resulting in the inhibition of key signaling cascades in malignant tumor cells. Among the different classes of promising molecular targets for therapeutic purposes in ATC are heat shock proteins (HSP) and NF-κB [2].

Hsp90 functions as a molecular chaperone by binding to different cellular proteins (protein kinases,

How to cite this article: Todorović L, Stamenković G, Vučetić Tadić B, Umezawa K, Božović A, YamashitaS, Stanojević B.Synergistic effect of 17-allylamino-17-demethoxygeldanamycin with dehydroxymethylepoxyquinomicin on the human anaplastic thyroid carcinoma cell line KTC2. Arch Biol Sci. 2021;73(1):31-8.

transcription factors, etc.), and supporting their proper folding, stability and function. Many of these client proteins are involved in cell proliferation, differentiation, invasion, metastasis and drug resistance and are thus frequently mutated or overexpressed in cancer. Intact Hsp90 chaperone function is what cancer cells are particularly dependent on, since inhibition of Hsp90 leads to ubiquitin-proteasome degradation of its client proteins. Moreover, enhanced Hsp90 affinity for mutated or functionally deregulated target proteins in cancer cells has been observed and documented. This explains why Hsp90, which is often overexpressed in cancer, plays a critical role in tumor cell growth and/or survival [3-5]. Hsp90 operates as an ATP-binding system. The benzoquinone ansamycin antibiotic geldanamycin and its less toxic analog with improved bioavailability, 17-AAG, were found to inhibit Hsp90 multi-chaperone complex formation by docking into the ATP-binding site in the N-terminal domain of this protein [6,7]. Although Hsp90 is expressed both in normal and tumor cells, it was determined that tumor cells harbor a finely-tuned Hsp90 fraction that exists in an altered conformation, with a 100-fold higher binding affinity for 17-AAG than Hsp90 derived from normal cells [8,9]. This, as well as the fact that Hsp90 regulates multiple proteins involved in different processes during cancer development and progression, makes it a unique anticancer target. Cytotoxic activities of 17-AAG were demonstrated in different thyroid cancer cell lines among others, including the anaplastic thyroid cancer cell lines ARO, FRO, ACT1, 8505C and CAL62 [10-13]. A number of phase I, II and III clinical trials of 17-AAG have been conducted so far, as summarized in [14]. Data from these clinical trials show that 17-AAG can be given safely at biologically active dosages, with mild toxicity. Although phase I and II clinical trials of 17-AAG as a single agent did not demonstrate considerable clinical efficiency, the combination of 17-AAG with other anticancer drugs showed more promise regarding the clinical benefit for cancer patients [14].

NF-κB is a ubiquitously expressed proinflammatory transcription factor that regulates the expression of a number of genes involved in inflammation as well as in cell proliferation, apoptosis, invasion, angiogenesis and metastasis. NF-κB is typically a heterodimer comprised of Rel family proteins p50 and p65. These dimers are usually located in the cytoplasm in an inactive form as a result of association with inhibitory factors, IκB proteins. A number of extracellular signals can lead to phosphorylation and degradation of IκB, resulting in NF-κB activation and translocation to the nucleus, where it binds to specific promoters and regulates target gene expression [15]. As an antiapoptotic factor, NF-κB is one of the major reasons of resistance to chemotherapy, and it crucially affects the outcomes of cancer treatments [16]. ATC often exhibits constitutive and strong activation of NF-κB, which is extensively correlated with resistance to therapy and the aggressiveness of ATC [17]. Furthermore, a recent study identified NF-κB signaling pathways to be among the key pathways that regulate cancer stem cell properties in ATC [18]. An NF-κB inhibitor – DHMEQ, a derivative of the antibiotic epoxyquinomicin C, has been found to inhibit tumor necrosis factor (TNF)-induced activation of NF-κB by suppressing NF-κB nuclear translocation, thus promoting apoptosis in a variety of cancer cells [19-22], including anaplastic thyroid carcinoma cells [23].

The aim of this study was to determine whether the combined treatment with 17-AAG and DHMEQ displays improved antitumor activity over either agent alone in the anaplastic thyroid carcinoma cell line KTC2. To our knowledge, this is the first time that the combined effect of these two agents is investigated in the anaplastic thyroid carcinoma cells.

MATERIALS AND METHODS

Reagents

Both 17-AAG and DHMEQ were dissolved in 99.9% dimethyl sulfoxide (DMSO) (Wako Chemicals, Osaka, Japan) at stock concentrations of 1 mM and 10 mg/mL, respectively, and stored at -20°C. Primary antibodies were obtained from the following sources: Bcl-XL polyclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA); XIAP polyclonal antibody, PARP-1 polyclonal antibody, and cleaved caspase 3 polyclonal antibody were all from Cell Signaling Technology (Danvers, MA, USA). The polyclonal antibody to cIAP-2 was from R&D Systems (Minneapolis, MN, USA). Secondary antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA).

Cell culture

The human anaplastic thyroid carcinoma cell line, KTC-2, derived from pleural effusion metastasis, with no TP53 mutation, was provided by the Department of Molecular Medicine, Medical School, Nagasaki University, Japan. Cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% fetal bovine serum (FBS) and 1% (w/v) penicillin/streptomycin in a 5% CO_2 humidified atmosphere at 37°C. Primary human thyroid cell culture was established as described [24] and maintained in a Dulbecco's Modified Eagle Medium (DMEM):F12 mixture (1:2) supplemented with 3% FBS and 1% (w/v) penicillin/streptomycin [all reagents were obtained from Sigma (St. Louis, MO, USA)].

Cell survival assay

Cell suspensions (100 µL, 3000 cells per well) were added to each well of 96-well plate flat-bottom microtiter plates (NalgeNunc International, Tokyo, Japan) in RPMI 1640 containing 5% FBS, and incubated for 24 h before treatment. Solutions, in increasing concentrations ranging from 1 nM to 1 µM of 17-AAG and with DHMEQ at a dose range of 0.1 µg/mL to 20 µg/mL, respectively, were added to each well in 11 µL medium. The control wells contained DMSO at a final concentration of 0.1%. After incubation, a water-soluble tetrazolium salt (WST)-based assay was performed as follows: 10 µL of the cell counting kit solution (CCK-8, Dojin, Osaka, Japan) were added to each well and incubated for 1 h at 37°C. Optical densities were measured at 450 nm in a microplate reader. Thus, the drug concentrations of 100 nM of 17-AAG and 2-10 µg/mL DHMEQ were determined as the most effective after 24, 48 and 72 h of treatment.

Analysis of the combined drug effects

Drug synergy was determined by the combination-index and isobologram analyses. Briefly, the combination-index (CI) is a quantitative representation of the degree of drug interaction calculated by the following equation $(CI) = (D)1/(D\chi)1 + (D)2/(D\chi)2$ using the CalcuSyn software (Biosoft, Ferguson, MO, USA) where $(D\chi)1$ and $(D\chi)2$ are the concentrations of each drug alone, which exert χ% effect while (D)1 and (D)2 are the concentrations of drugs in combination, which elicit the same effect. The fraction affected (Fa) represents the fraction affected by a given drug concentration; the evolution of drug interactions can be assessed by the Fa-CI plot. On the basis of the dose-response curves obtained from the cell survival assay for KTC2 treated with the inhibitors, alone or in combination, the CI values were generated over the range of fraction affected (Fa) levels from 5%-95% growth inhibition. Synergism, additivity and antagonism are defined as CI<1, CI=1 and CI>1, respectively [25].

Preparation of cell extracts and Western blot

After the indicated treatment duration, the treated cells were washed twice in an ice-cold phosphate-buffered saline (PBS), collected in 1 mL of PBS and centrifuged at 1000 ×*g* for 3 min. The pellets were resuspended in 200 µL of lysis buffer (Cell Signaling Technology, Beverly, MA, USA) containing protease and phosphatase inhibitors and incubated for 15 min on ice. Lysates were then centrifuged for 15 min at 25000 ×*g* at 4°C, and the supernatants were stored at -80°C. Protein concentrations were determined with the bicinchoninic acid (BCA) assay kit (Sigma-Aldrich, St. Louis, MO, USA).

Western blotting analysis was performed according to standard procedures as described previously [26]. Proteins were resolved by the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA), and subjected to immunoblotting with appropriate primary and secondary antibodies. The antigen-antibody complexes were visualized using chemiluminescent reagents (Amersham, Arlington Height, IL, USA). Densitometry analysis was performed using ImageJ software [27].

RESULTS

Cytotoxic effects of 17-AAG and DHMEQ in anaplastic thyroid carcinoma cells

The combined treatment of 17-AAG and DHMEQ strongly enhanced the growth-inhibitory effect in KTC2 cells compared to each single drug treatment. This effect was already evident after 24 h of treatment, as shown in Fig. 1A. The inhibitory effect was increased

even more after 48 h of treatment (Fig. 1B) and by 72 h, no cells survived at any of the 4 indicated dose combinations (data not shown). In contrast, cultured primary thyrocytes showed substantially lower sensitivity to combined treatment, and no significant effect on primary cell survival was observed until the DHMEQ dose was increased to 10 μg/mL (Fig. 1C and 1D).

17-AAG and DHMEQ combined drug effect

The combination index method showed a synergistic antiproliferation effect of 17-AAG and DHMEQ in KTC2 cells, which was observed at all 4 dose combinations (Fig. 2).

Apoptotic changes in 17-AAG and DHMEQ treated thyroid cancer cells

Immunoblot analysis showed that the combined treatment triggered the cleavage of proapoptotic molecules, caspase 3 and PARP-1, in KTC2 cells. Higher levels of cleaved PARP-1 and caspase 3 appeared after 48 h of combined treatment with 100 nM of 17-AAG and 2 μg/mL of DHMEQ, and this trend was also evident when higher doses (4, 8 and 10 μg/mL) of DHMEQ were used. Also, the combined treatment markedly reduced the expression of inhibitors of apoptosis Bcl-XL, XIAP and cIAP-2, when compared to the effect observed with either agent alone (Fig.3).

DISCUSSION

After promising preclinical studies of 17-AAG in various cancer cell lines and xenograft models [12,28-30], single agent clinical studies of this compound had limited success [14,29]. On the other hand, studies of 17-AAG in combination with other anticancer drugs showed more clinical benefit for cancer patients, despite drawbacks such as low water solubility and high hepatotoxicity, which could be addressed in the future by

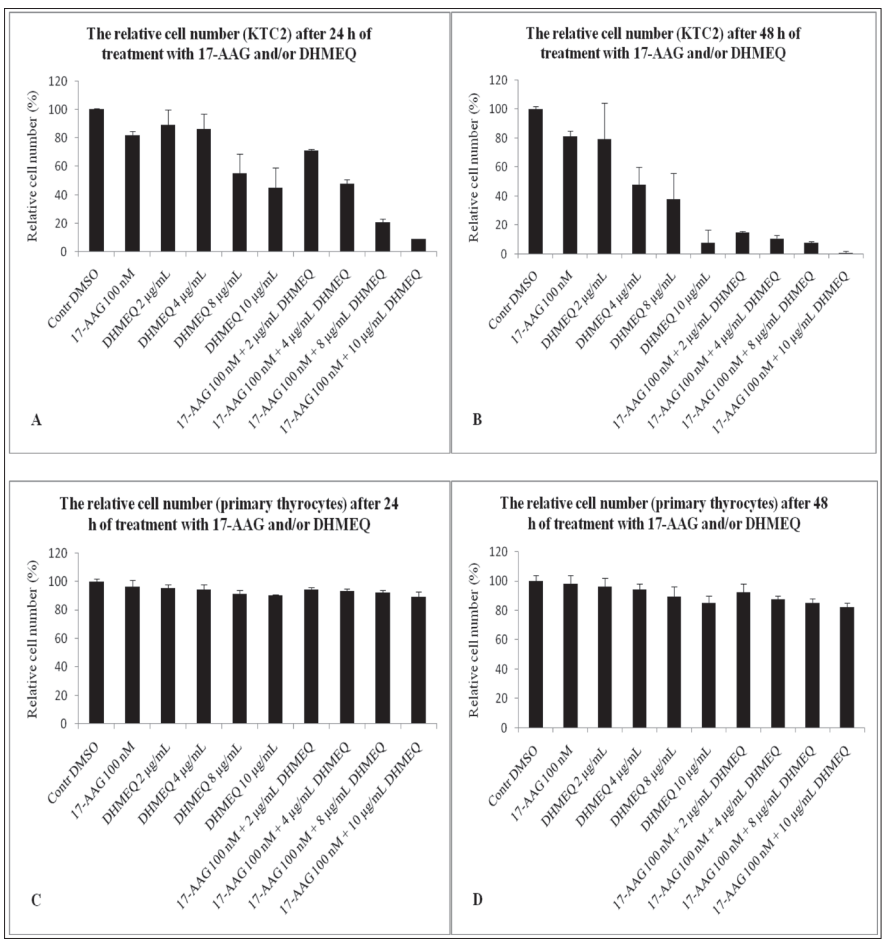


Fig. 1. Cell viability and synergy achieved by the combination of 17-AAG and DHMEQ in KTC2 cells and primary thyrocytes. Cells were treated with different concentrations of single or both drugs, and viabilities were determined by the WST-based assay as explained in the Materials and Methods. **A, B** – viability of KTC2 cells treated with the indicated concentrations of 17-AAG and DHMEQ for 24 h and 48 h, respectively; **C, D** – viability of cultured primary thyrocytes treated with the indicated concentrations of 17-AAG and DHMEQ for 24 h and 48 h, respectively. 17-AAG – 17-allylamino-17-demethoxygeldanamycin; DHMEQ – dehydroxymethylepoxyquinomicin; Bars, mean±SD from three replicates.

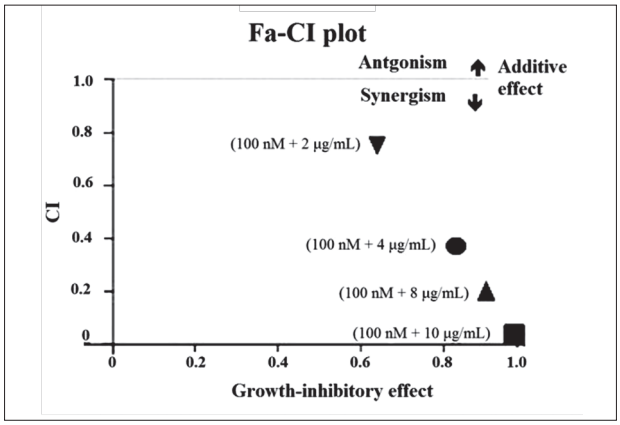
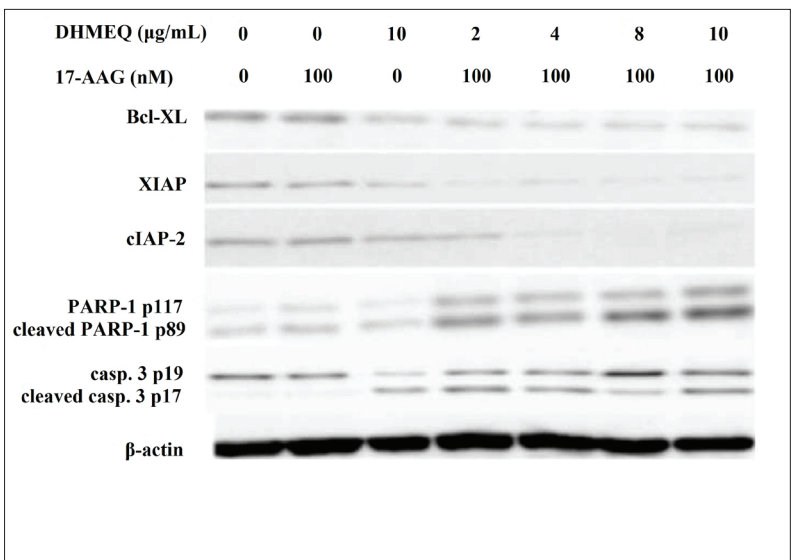


Fig. 2. Combination index analysis of 17-AAG/DHMEQ. The straight line (additivity line) is the locus of all dose pairs that should give the same effect. A dose pair below the line is synergistic, a dose pair on the line is additive, a dose pair above the line is antagonistic. 17-AAG was found to potently synergize with DHMEQ at 4 indicated dose pairs after 24, 48 and 72 h of treatment. The growth-inhibitory effect was measured by the WST-based assay. The figure represents the analysis after 24 h of treatment.



DHMEQ (μg/mL)	0	0	10	2	4	8	10
17-AAG (nM)	0	100	0	100	100	100	100
Bcl-XL	0.533	0.515	0.289	0.227	0.196	0.242	0.183
XIAP	0.274	0.219	0.108	0.030	0.051	0.036	0.032
cIAP-2	0.254	0.231	0.172	0.127	0.035	0.006	0.012
PARP-1 p117	0.047	0.065	0.032	0.144	0.137	0.166	0.192
cleav. PARP-1 p89	0.154	0.157	0.103	0.328	0.269	0.463	0.386
cas.3 p19	0.449	0.342	0.107	0.261	0.302	0.495	0.339
cleav. cas.3 p17	0.038	0.039	0.236	0.325	0.268	0.193	0.264

Fig. 3. Western blot analysis of the effect of the drugs on changes of pro- and anti-apoptotic factors. Cells were treated with the indicated concentrations of 17-AAG and DHMEQ for 48 h-and whole-cell lysates were examined by Western blotting. β-actin was used as the loading control. The densitometry results are presented in the table below the Western blot. The expression levels of target proteins are normalized to β-actin.

implementation of nanomaterials-based drug delivery carriers [14]. However, more studies in various types of malignant tumors are needed to assess the real clinical potential of this Hsp90 inhibitor, as well as to identify other agents capable of improving its anticancer effects. DHMEQ, an NF-κB inhibitor, was shown to be a potent chemo- and immunosensitizing agent which, in combination with cytotoxic therapeutics resulted in significant reversal of resistance and tumor cell death in various tumors, including anaplastic thyroid carcinoma [20,31-36]. To our knowledge, no studies of the joint effect of these two compounds in cancer cells have been conducted so far.

In the present study, we analyzed the effects of the combined treatment of 17-AAG and DHMEQ in human anaplastic thyroid carcinoma cell line KTC2, as compared to the effect of either agent alone. Consistent with previous studies, both DHMEQ and 17-AAG alone displayed a cytotoxic effect on thyroid cancer cells [10-12,23,36]. The combined treatment, however, already after the first 24 h strongly enhanced the growth-inhibitory effect compared with the single agent treatment. This effect was increased even more after 48 h, and by 72 h no cells survived. Moreover, we observed a synergistic antiproliferative effect of the combined treatment with all 4 dose combinations of the two agents. Furthermore, we established that the combined treatment of 17-AAG and DHMEQ potentiated apoptosis, this being probably one of the mechanisms for controlling tumor cell growth. Apoptosis induction was confirmed by cleavage of some of the characteristic indices of apoptosis, such as caspase 3 (the key executioner of apoptosis) and PARP-1 (the main cleavage target of caspase 3). Also, the combined treatment markedly reduced the expression of the antiapoptotic protein Bcl-xL, as well as the expression of cIAP-2 and XIAP, which are members of the inhibitors of apoptosis family (IAPs) and are known to be under transcriptional regulation of NF-κB [37]. Consistent with previous studies, in our study the treatment

with 17-AAG alone was not associated with caspase 3 cleavage nor significant changes of expression of Bcl-XL, XIAP and cIAP-2 [11,12] while DHMEQ treatment was most probably the result of NF-κB inhibition [22, 23]. However, the changes in expression of the apoptotic markers were more prominent when the combined treatment of 17-AAG and DHMEQ was applied. The higher levels of cleaved PARP-1 and caspase 3 appeared after 48 h of combined treatment and were evident at all dose combinations. While some studies [22,23] have reported that the changes in expression level of Bcl-XL in ATC cells treated with DHMEQ were not as prominent as the suppression of IAPs, we showed that the combined treatment markedly reduced the expression level of this antiapoptotic protein, which was also accompanied by a significant reduction in the expression level of XIAP and cIAP-2. Taken together, these results are in agreement with other studies reporting that DHMEQ can act synergistically with different therapeutic agents in promoting apoptosis in various cancer cell lines [33,38-41]. In contrast to carcinoma cells, in primary thyrocytes the combination treatment was accompanied by more than 90% cell survival rate. This is in accordance with previous studies in which it was reported that cancer cells are significantly more sensitive to Hsp90 inhibition than non-transformed cells, as reviewed in [5,29], and studies indicating that normal thyroid epithelial cells, compared to cancer cells, show relative resistance to DHMEQ-induced apoptosis [23].

In light of the fact that dose-related toxicities were among the drawbacks for a more successful treatment with 17-AAG in clinical studies, another interesting finding of our study is that potent growth inhibition was achieved by using an overall lower dose of 17-AAG (when combined with various doses of DHMEQ) compared to doses used thus far [10-12,42,43]. This might indicate a potential strategy to decrease 17-AAG off-site toxicity in *in vivo* studies, justifying further investigations.

Finally, we would like to emphasize some of the limitations of the present study. The first is that the study included only one ATC cell line; it would be prudent to repeat the experiments on several other ATC cell lines. We also did not address the effect of 17-AAG and DHMEQ on the activity of their targets, Hsp90 and NF-κB, respectively, in order to confirm their interaction. Since quantitative Western blotting experiments using untested assumptions of proportionality for normalization can be misleading (as described in [44]), our results of the expression of apoptosis markers should be taken with caution when interpreting the results related to the effect of the drugs on apoptosis.

CONCLUSION

Herein we provide the first *in vitro* evidence that the combination of Hsp90 and NF-κB inhibitors, 17-AAG and DHMEQ respectively, working in a synergistic manner, strongly inhibit cancer cell growth and potentiate apoptosis in human anaplastic thyroid carcinoma cells. Further *in vitro* and *in vivo* studies of the joint action of these two compounds are needed in order to clarify the underlying mechanisms, as well as to provide an estimate of their potential for translational applications in anaplastic thyroid carcinoma patients.

Funding: This study was financially supported by the Nagasaki University Global COE Program.

Acknowledgments: The authors would like to thank Dr Vladimir Saenko from Atomic Bomb Disease Institute, Nagasaki University, for providing valuable suggestions and comments.

Author contributions: LT – interpretation of the results, preparation of the manuscript; GS – preparation of the manuscript; BVT – interpretation of the results; KU – critical review of the manuscript; AB – interpretation of the results; SY – critical review of the manuscript; BS –design of the study, experimental work, interpretation of the results, preparation of the manuscript.

Conflicts of interest disclosure: The authors confirm that there are no conflicts of interests.

REFERENCES

1. Chintakuntlawar AV FR, Kasperbauer JL, Bible KC. Diagnosis and Management of Anaplastic Thyroid Cancer. Endocrinol Metab Clin North Am. 2019;48(1):269-84.
2. Shikha Saini KT, Ajay V Maker, Kenneth D Burman, Bellur S Prabhakar Therapeutic advances in anaplastic thyroid cancer: a current perspective. Mol Canc. 2018;17(1):154. https://doi.org/10.1186/s12943-018-0903-0
3. Pearl LH, Prodromou C, Workman P. The Hsp90 molecular chaperone: an open and shut case for treatment. Biochem J. 2008;410(3):439-53. https://doi.org/10.1042/bj20071640
4. Workman P. Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. Cancer Lett. 2004;206(2):149-57. https://doi.org/10.1016/j.canlet.2003.08.032

5. Chiosis G, Neckers L. Tumor selectivity of Hsp90 inhibitors: the explanation remains elusive. ACS Chem Biol. 2006;1(5):279-84. https://doi.org/10.1021/cb600224w
6. Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A. 1994;91(18):8324-8. https://doi.org/10.1073/pnas.91.18.8324
7. Hadden MK, Lubbers DJ, Blagg BS. Geldanamycin, radicicol, and chimeric inhibitors of the Hsp90 N-terminal ATP binding site. Curr Top Med Chem. 2006;6(11):1173-82. https://doi.org/10.2174/156802606777812031
8. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, Burrows FJ. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature. 2003;425(6956):407-10. https://doi.org/10.1038/nature01913
9. Moulick K, Ahn JH, Zong H, Rodina A, Cerchietti L, Gomes DaGama EM, Caldas-Lopes E, Beebe K, Perna F, Hatzi K, Vu LP, Zhao X, Zatorska D, Taldone T, Smith-Jones P, Alpaugh M, Gross SS, Pillarsetty N, Ku T, Lewis JS, Larson SM, Levine R, Erdjument-Bromage H, Guzman ML, Nimer SD, Melnick A, Neckers L, Chiosis G. Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. Nat Chem Biol. 2011;7(11):818-26. https://doi.org/10.1038/nchembio.670
10. Kim SH, Kang JG, Kim CS, Ihm SH, Choi MG, Yoo HJ, Lee SJ. 17-Allylamino-17-demethoxygeldanamycin and Herbimycin A Induce Cell Death by Modulating beta-Catenin and PI3K/AKT Signaling in FRO Anaplastic Thyroid Carcinoma Cells. Anticancer Res. 2015;35(10):5453-60. https://doi.org/10.1007/s12020-014-0371-2
11. Kim SH, Kang JG, Kim CS, Ihm SH, Choi MG, Yoo HJ, Lee SJ. The effect of 17-allylamino-17-demethoxygeldanamycin alone or in combination with paclitaxel on anaplastic thyroid carcinoma cells. Endocrine. 2015;48(3):886-93. https://doi.org/10.1007/s12020-014-0371-2
12. Braga-Basaria M, Hardy E, Gottfried R, Burman KD, Saji M, Ringel MD. 17-Allylamino-17-demethoxygeldanamycin activity against thyroid cancer cell lines correlates with heat shock protein 90 levels. J Clin Endocrinol Metab. 2004;89(6):2982-8. https://doi.org/10.1210/jc.2003-031767
13. White PT, Subramanian C, Zhu Q, Zhang H, Zhao H, Gallagher R, Timmermann BN, Blagg BS, Cohen MS. Novel HSP90 inhibitors effectively target functions of thyroid cancer stem cell preventing migration and invasion. Surgery. 2016;159(1):142-51. https://doi.org/10.1016/j.surg.2015.07.050
14. Talaei S, Mellatyar H, Asadi A, Akbarzadeh A, Sheervalilou R, Zarghami N. Spotlight on 17-AAG as an Hsp90 inhibitor for molecular targeted cancer treatment. Chem Biol Drug Des. 2019;93(5):760-86. https://doi.org/10.1111/cbdd.13486
15. Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta. 2010;1799(10-12):775-87. https://doi.org/10.1016/j.bbagrm.2010.05.004
16. Namba H, Saenko V, Yamashita S. Nuclear factor-kB in thyroid carcinogenesis and progression: a novel therapeutic target for advanced thyroid cancer. Arq Bras Endocrinol Metabol. 2007;51(5):843-51. https://doi.org/10.1590/s0004-27302007000500023
17. Pacifico F, Leonardi A. Role of NF-kappaB in thyroid cancer. Mol Cell Endocrinol. 2010;321(1):29-35.
18. Shiraiwa K, Matsuse M, Nakazawa Y, Ogi T, Suzuki K, Saenko V, Xu S, Umezawa K, Yamashita S, Tsukamoto K, Mitsutake N. JAK/STAT3 and NF-kappaB Signaling Pathways Regulate Cancer Stem-Cell Properties in Anaplastic Thyroid Cancer Cells. Thyroid. 2019;29(5):674-82. https://doi.org/10.1089/thy.2018.0212
19. Umezawa K, Chaicharoenpong C. Molecular design and biological activities of NF-kappaB inhibitors. Mol Cells. 2002;14(2):163-7.
20. Castro-Gamero AM, Borges KS, da Silva Silveira V, Lira RC, de Paula Gomes Queiroz R, Valera FC, Scrideli CA, Umezawa K, Tone LG. Inhibition of nuclear factor-kappaB by dehydroxymethylepoxyquinomicin induces schedule-dependent chemosensitivity to anticancer drugs and enhances chemoinduced apoptosis in osteosarcoma cells. Anticancer Drugs. 2012;23(6):638-50. https://doi.org/10.1097/cad.0b013e328350e835
21. Horiguchi Y, Kuroda K, Nakashima J, Murai M, Umezawa K. Antitumor effect of a novel nuclear factor-kappa B activation inhibitor in bladder cancer cells. Expert Rev Anticancer Ther. 2003;3(6):793-8. https://doi.org/10.1586/14737140.3.6.793
22. Nishimura D, Ishikawa H, Matsumoto K, Shibata H, Motoyoshi Y, Fukuta M, Kawashimo H, Goto T, Taura N, Ichikawa T, Hamasaki K, Nakao K, Umezawa K, Eguchi K. DHMEQ, a novel NF-kappaB inhibitor, induces apoptosis and cell-cycle arrest in human hepatoma cells. Int J Oncol. 2006;29(3):713-9. https://doi.org/10.3892/ijo.29.3.713
23. Starenki DV, Namba H, Saenko VA, Ohtsuru A, Maeda S, Umezawa K, Yamashita S. Induction of thyroid cancer cell apoptosis by a novel nuclear factor kappaB inhibitor, dehydroxymethylepoxyquinomicin. Clin Cancer Res. 2004;10(20):6821-9. https://doi.org/10.1158/1078-0432.ccr-04-0463
24. Kawabe Y, Eguchi K, Shimomura C, Mine M, Otsubo T, Ueki Y, Tezuka H, Nakao H, Kawakami A, Migita K, Yamashita S, Matsunga M, Ishikawa N, Ito K, Nagataki S. Interleukin-1 production and action in thyroid tissue. J Clinic Endocrinol Metabol. 1989;68(6):1174-83. https://doi.org/10.1210/jcem-68-6-1174
25. Chou TC TP. Analysis of combined drug effects: a new look at a very old problem. Trends Pharmacol Sci. 1983;4:450-54.
26. Liu D, Hou P, Liu Z, Wu G, Xing M. Genetic alterations in the phosphoinositide 3-kinase/Akt signaling pathway confer sensitivity of thyroid cancer cells to therapeutic targeting of Akt and mammalian target of rapamycin. Cancer Res. 2009;69(18):7311-9. https://doi.org/10.1158/0008-5472.can-09-1077
27. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012;9(7):671-75. https://doi.org/10.1038/nmeth.2089
28. Liu R, Blower PE, Pham AN, Fang J, Dai Z, Wise C, Green B, Teitel CH, Ning B, Ling W, Lyn-Cook BD, Kadlubar FF, Sadee W, Huang Y. Cystine-glutamate transporter SLC7A11 mediates resistance to geldanamycin but not to 17-(allylamino)-17-demethoxygeldanamycin. Mol Pharmacol. 2007;72(6):1637-46. https://doi.org/10.1124/mol.107.039644

29. Shrestha L, Bolaender A, Patel HJ, Taldone T. Heat Shock Protein (HSP) Drug Discovery and Development: Targeting Heat Shock Proteins in Disease. Curr Top Med Chem. 2016;16(25):2753-64. https://doi.org/10.2174/1568026616666160413141911
30. Xu Y, Zhu Q, Chen D, Shen Z, Wang W, Ning G, Zhu Y. The HSP90 inhibitor 17-AAG exhibits potent antitumor activity for pheochromocytoma in a xenograft model. Tumour Biol. 2015;36(7):5103-8. https://doi.org/10.1007/s13277-015-3162-3
31. Katsman A, Umezawa K, Bonavida B. Chemosensitization and immunosensitization of resistant cancer cells to apoptosis and inhibition of metastasis by the specific NF-kappaB inhibitor DHMEQ. Curr Pharm Des. 2009;15(7):792-808. https://doi.org/10.2174/138161209787582156
32. Heavey S, Godwin P, Baird AM, Barr MP, Umezawa K, Cuffe S, Finn SP, O'Byrne KJ, Gately K. Strategic targeting of the PI3K-NFkappaB axis in cisplatin-resistant NSCLC. Cancer Biol Ther. 2014;15(10):1367-77. https://doi.org/10.4161/cbt.29841
33. Ramos PMM, Pezuk JA, Castro-Gamero AM, Oliveira HF, Scrideli CA, Umezawa K, Tone LG. Antineoplastic Effects of NF-kappaB Inhibition by DHMEQ (Dehydroxymethylepoxyquinomicin) Alone and in Co-treatment with Radio-and Chemotherapy in Medulloblastoma Cell Lines. Anticancer Agents Med Chem. 2018;18(4):541-49. https://doi.org/10.2174/1871520617666171113151335
34. Ryan SL, Beard S, Barr MP, Umezawa K, Heavey S, Godwin P, Gray SG, Cormican D, Finn SP, Gately KA, Davies AM, Thompson EW, Richard DJ, O'Byrne KJ, Adams MN, Baird AM. Targeting NF-kappaB-mediated inflammatory pathways in cisplatin-resistant NSCLC. Lung Cancer. 2019;135:217-27. https://doi.org/10.1016/j.lungcan.2019.07.006
35. Pushkarev VV, Starenki DV, Pushkarev VM, Kovzun OI, Tronko MD. Inhibitor of the transcription factor NF-kappaB, DHMEQ, enhances the effect of paclitaxel on cells of anaplastic thyroid carcinoma in vitro and in vivo. Ukr Biochem J. 2015;87(3):63-74. https://doi.org/10.15407/ubj87.03.063
36. Meng Z, Mitsutake N, Nakashima M, Starenki D, Matsuse M, Takakura S, Namba H, Saenko V, Umezawa K, Ohtsuru A, Yamashita S. Dehydroxymethylepoxyquinomicin, a novel nuclear Factor-kappaB inhibitor, enhances antitumor activity of taxanes in anaplastic thyroid cancer cells. Endocrinology. 2008;149(11):5357-65. https://doi.org/10.1210/en.2008-0279
37. Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene. 1999;18(49):6910-24. https://doi.org/10.1038/sj.onc.1203238
38. Lampiasi N, Azzolina A, Umezawa K, Montalto G, McCubrey JA, Cervello M. The novel NF-kappaB inhibitor DHMEQ synergizes with celecoxib to exert antitumor effects on human liver cancer cells by a ROS-dependent mechanism. Cancer Lett. 2012;322(1):35-44. https://doi.org/10.1016/j.canlet.2012.02.008
39. Togano T, Nakashima M, Watanabe M, Umezawa K, Watanabe T, Higashihara M, Horie R. Synergistic effect of 5-azacytidine and NF-kappaB inhibitor DHMEQ on apoptosis induction in myeloid leukemia cells. Oncol Res. 2012;20(12):571-7. https://doi.org/10.3727/096504013x13775486749371
40. Brassesco MS, Roberto GM, Morales AG, Oliveira JC, Delsin LE, Pezuk JA, Valera ET, Carlotti CGJr, Rego EM, de Oliveira HF, Scrideli CA, Umezawa K, Tone LG. Inhibition of NF- kappa B by Dehydroxymethylepoxyquinomicin Suppresses Invasion and Synergistically Potentiates Temozolomide and gamma -Radiation Cytotoxicity in Glioblastoma Cells. Chemother Res Pract. 2013;2013:593020. https://doi.org/10.1155/2013/593020
41. Watanabe M, Umezawa K, Higashihara M, Horie R. Combined inhibition of NF-kappaB and Bcl-2 triggers synergistic reduction of viability and induces apoptosis in melanoma cells. Oncol Res. 2013;21(4):173-80. https://doi.org/10.3727/096504014x13887748696707
42. Park JW, Yeh MW, Wong MG, Lobo M, Hyun WC, Duh QY, Clark OH. The heat shock protein 90-binding geldanamycin inhibits cancer cell proliferation, down-regulates oncoproteins, and inhibits epidermal growth factor-induced invasion in thyroid cancer cell lines. J Clin Endocrinol Metab. 2003;88(7):3346-53. https://doi.org/10.1210/jc.2002-020340
43. Wang C, Zhang R, Tan J, Meng Z, Zhang Y, Li N, Wang H, Chang J, Wang R. Effect of mesoporous silica nanoparticles coloading with 17AAG and Torin2 on anaplastic thyroid carcinoma by targeting VEGFR2. Oncol Rep. 2020;43(5):1491-502. https://doi.org/10.3892/or.2020.7537
44. Butler TAJ, Paul JW, Chan E-C, Smith R, Tolosa JM. Misleading Westerns: Common Quantification Mistakes in Western Blot Densitometry and Proposed Corrective Measures. BioMed Research International. 2019;2019:5214821. https://doi.org/10.1155/2019/5214821