Research Article

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Archives in Cancer Research
2254-6081

2023
Vol.11 No. 1: 101

The Suppressive Effects of Cytotoxic Agents on Lymphoma Cell Proliferation and Tumor Growth

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Citation: Vasilevich NI, Sun L, Yang M, Xiao H, Yang J, et al. (2023) The Suppressive Effects of Cytotoxic Agents on Lymphoma Cell Proliferation and Tumor Growth. Archives Can Res, Vol.11 No. 1: 101.

Abstract

Objective: Ansamitocin P3 (AP3) and paclitaxel as tubulin inhibitors and camptothecin (CPT) as a Topo Ⅰ inhibitor are considered to be among the most active natural products. We investigate the roles and mechanisms of AP3, paclitaxel and CPT in inducing apoptosis in human histiocytic lymphoma U937 cells.

Methods and materials: In vitro studies included CCK8 assay to estimate antiproliferative activity and flow cytometry to analyze apoptosis of U937 cells. qRT-PCR was revealed the change in gene expression leading to apoptosis. In vivo experiments were performed with xenograft tumor model using five weeks-old female SCID mice.

Results: CCK8 assay exhibits AP3 as a great antiproliferative activity compound with a half-maximal inhibitory concentration (IC_{50}) at 0.18 ± 0.04 nM, while the IC_{50}s of CPT and paclitaxel were at 25.09 ± 2.64 and 6.06 ± 1.24 nM respectively. Apoptosis analysis indicated that the number of both early and late apoptotic cells increased significantly after treatment with each of three abovementioned biomolecules. Cell cycle analysis suggested that while both AP3 and paclitaxel arrest cells in the G2/M Phase at middle or high concentrations, CPT arrests cells in G2/M phase at low concentration and in the S phase at high concentration. Transcritome's qRT-PCR found that AP3 and paclitaxel induce apoptosis downregulating the expression of PCNA and BCL-2, and besides that, cells treated with CPT showed upregulated P21 but downregulated BCL-2 expression. The data obtained using xenograft tumor model showed that AP3 greatly inhibits tumor growth with little side effect, which confirms high in vivo anti-tumor activity of this compound.

Conclusion: The obtained results suggest the strongest antitumor activity of AP3 on lymphoma U937 cells and its great potential in tumor-targeted therapy.

Keywords: Lymphoma cells; Ansamitocin P3; Paclitaxel; Camptothecin; Apoptosis; Tumor growth

Received: 3-Jan-2023, Manuscript No. ipacr-22-13343; **Editor assigned:** 06-Jan-2023, Preqc No. PQ-ipacr-22-13343; **Reviewed:** 20-Jan-2023, QC No ipacr-22-13343; **Revised:** 23-Jan-2023, Manuscript No. ipacr-22-13343 (R); **Published:** 30-Jan-2023, DOI: 10.36648/2254-6081-11.1-101

Introduction

Lymphoma is a malignant tumor which ranks among the ten most prevalent cancers worldwide. It is more likely to occur in lymph nodes, however, due to flow of the lymphatic system it can affect almost any tissue and organ in the body [1, 2]. In recent years, extensive research efforts were focused on tumor-targeted therapy designed to attack tumors with optimal efficacy and little damage to normal cells [3]. Antibody-drug conjugate (ADC) containing antibodies specific to tumor cells and a cytotoxic payload is an example of such approach. Selection of small molecules with extremely low value of half maximal inhibitory concentration (IC_{50}) is the key aspect of the effectiveness of ADC treatment [4, 5].

In the long history of antitumor research, a lot of natural products extracted from plants and microorganisms were found to show strong antitumor activity [6, 7]. Among the main targets for anticancer therapy are the microtubules (MTs), which play an essential role in mitosis [8-10]. Ansamitocin P3 (AP3), which is

an analogue of maytansine isolated from Nocardia species [11], binds to tubulin at the vinblastin site and disrupts the assembly of microtubules [12]. After treatment with AP3, cells are arrested in mitotic phase and then apoptosis is induced. In spite of strong cytotoxicity, AP3 has not been approved for clinical use due to its severe side effects and narrow therapeutic spectrum [13].

Paclitaxel isolated from the Pacific yew tree species Taxus brevifolia has been approved by FDA for treatment of many kinds of cancers including ovarian and breast cancers and it is one of the most widely used drugs [14-18]. As a microtubule-stabilizing drug, it can prevent chromosome segregation, cause mitotic arrest and induce apoptosis of cells [19, 20]. However, the high toxicity and low solubility of paclitaxel limit its application. In order to improve the solubility and safety of paclitaxel nanotechnology can be used [21, 22].

Camptothecin (CPT), a pentacyclic alkaloid extracted from Camptotheca acuminata, demonstrates a good antitumor effect and has been used in anti-cancer therapy since 1994 [23-25]. It disrupts the replication of DNA by inhibiting Topoisomerase Ⅰ, which then leads to apoptosis [26]. Irinotecan, a drug based on CPT, has been approved for treatment of several advanced cancers [27, 28], though this drug still suffers from high toxicity and poor solubility [24, 29].

A lot of biomolecules, such as ADCs, have shown potential for application as a part of tumor targeting conjugated molecules [30]. In this study, we compare the antitumor effects of AP3, paclitaxel and CPT on lymphoma U937 cells, investigate the potential mechanisms of apoptosis, and explore the inhibitory effect of AP3 in the xenograft tumor model. Our goal was to identify the therapeutic molecules suitable for targeting lymphoma or other cancer cells.

Materials and Method

Cell culture

Human histiocytic lymphoma U937 cells were purchased from Shanghai Cell Bank of the Chinese Academy of Sciences and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. All the reagents were purchased from Gibco (Thermo Fisher Scientific). Cells were incubated at 37°C and 5% CO_2 atmosphere.

Chemical compounds

Ansamitocin P3 (AP3) (purity > 98%), paclitaxel (purity 99.97%) and camptothecin (CPT) (purity 99.69%) were purchased from MCE (Med Chem Express, NJ, USA). PBS, RNase A were obtained from Solarbio (Solarbio Life Science, Beijing, China). Matrigel basement membrane matrix was supplied by BD Biosciences.

Cell proliferation

The survival rate was assessed in Cell Counting Kit-8 colorimetric (CCK8) assay (Dojindo, Kumamoto, Japan). The U937 cells were seeded into 96-well plates at 8×10^3 cells per well and incubated with different concentrations of AP3, paclitaxel and CPT (from 10^{-6} M to 10^{-13} M) for 72 hours. After adding 10 µl of CCK8 solution into each of the wells and incubating for two hours, the OD values were recorded at 450 nm with the microplate reader (Biotek, USA).

Cell cycle analysis

Cells were seeded into six-well plates with 2×10^5 cells per well, and treated with different concentrations of drugs for 24 hours. After being collected and washed with PBS, cells were fixed with 70% ethanol at -20 °C for from several hours to days, and centrifuged at 4000 rpm for 2 minutes. Cell pellets were resuspended in 500 µl PBS containing 0.25% Triton-X 100 and incubated on ice for 15 minutes. Then repeated centrifugation was performed, and 500 µl PBS with 10 µg/ml RNase A and 20 µg/ml PI were added. The resuspended cells were incubated in a dark place at room temperature for 30 minutes, and then cell cycle was analyzed by flow cytometer (Beckman, USA) with ModFit LT 5.0 software (Trial version).

Cell apoptosis analysis

Cells were seeded at 2×10^5 cells per well into six-well plates and treated with AP3, CPT and paclitaxel at different concentrations for 48 hours. After being collected and washed with PBS, they were suspended in 100 µl binding buffer and incubated with 5 µl FITC-AV and 5 µl PI (binding buffer, FITC-AV, PI were supplied with the detection kit) for 15 minutes. Finally, 400 µl binding buffer was added, and the samples were analyzed by flow cytometer using FlowJo V10 software (Trial version).

Gene expression analysis

Cell total RNA was isolated using Total RNA Kit (OMEGA, USA). The RNA was quantified by Nano Drop micro volume spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized from 1 µg RNA in 20 µl reaction using cDNA Synthesis kit (Genecopoeia, USA). 0.5 µl aliquotes of cDNA reaction mixture were included in 20 µl quantitative real-time PCR (qPCR) reaction with SYBR Green qPCR Mix 2.0 Kit (Genecopoeia, USA) as a template, and process was performed using CFX Connect Real-Time System (Bio-Rad, USA). Primers for the experiments were supplied by Sangon Biotech and their sequences are shown in (**Table 1**). All of the samples were tested in triplicates. The PCR process was set as follows: initial denaturation at 95°C for 30 seconds, and then 40 cycles of 10 seconds at 95°C denaturation and 30 seconds at 65°C for annealing/extension. Intensity of fluorescence was detected for each cycle. Produced data were analyzed with the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) with GAPDH as an internal control.

Table 1. Primer sequences of PCNA, P63, P21 and BCL-2.

	Primer sequence	
PCNA	Forward	TTAGCTCCAGCGGTGTAAAC
	Reverse	TTTGGACATACTGGTGAGGTTC
P63	Forward	CGTGTTATTGATGCTGTGCG
	Reverse	GAAGTCATTCCACTCATCTCGG
P21	Forward	TGTCACTGTCTTGTACCCTTG
	Reverse	GCGTTTGGAGTGGTAGAAATC
BCL-2	Forward	TTGTGGCCTTCTTTGAGTTCGGTG
	Reverse	GGTGCCGGTTCAGGTACTCAGTCA

Xenograft mouse tumor model

Five weeks-old female SCID mice were supplied by Beijing Vital River Laboratory Animal Technology (China). All mice were grown in SPF condition and obtained one week before the experiment to adapt to the new environment. Resuspended in PBS with Matrigel (ratio 1:1, total volume 100 µl) U937 cells (1×10^6) were injected subcutaneously into the right flank of each mouse. Tumor volume was estimated as 0.5 × Length × Width^2. After the tumor grew up to 70-120 mm^3, mice were divided into the control group and the experimental (AP3) group. Tail vein injection with AP3 (0.4 mg/kg, 100 µl) and 100 µl saline containing 10% ethanol was performed for mice of experimental (AP3) and control (saline) groups, respectively. Tumor measurements and bodyweights were recorded twice a week. The protocol was approved by Shanghai Medicilon IACUC (approval number: TEK2103P).

Statistical analysis

GraphPad 8.3.0. Software was used for analysis and evaluation of IC_{50}. All experiments were performed in triplicates and mean ± SD was calculated. t-test was used to estimate statistical significance and $p < 0.05$ was recognized as significant.

Results

The traditional chemotherapy displays their potent anti-tumor efficacy; however, the treatment is usually accompanied by severe toxic side effects. Thus, various strategies were applied to decrease the side effects of cytotoxic agents and increase their anti-tumor efficacy. As a part of our efforts to develop a receptor-targeting anti-tumor therapy via coupling the cytotoxic agents to peptide vehicles delivering them into tumor sites, we evaluated the potency of several compounds which were supposed to could be used in such drug conjugates.

Cell proliferation and citotoxicity

To compare cytotoxicity of the three abovementioned compounds, we used the CCK8 assay kit to estimate the U937 cells viability after 72-hour treatment with the compounds. Our results demonstrate that U937 cell proliferation and viability are highly compound- and dose-dependable (**Figure 1**). When the concentration of each of the compound increased and reached a critical point, the viability of U937 cells decreased crucially. In all groups of compounds, the cell viability accounted for 2% at their highest concentration. AP3 as well as CPT and paclitaxel demonstrated a strong antitumor activity with half-maximal proliferation inhibitory concentration (IC_{50}) in U937 cells 0.18 ± 0.04, 25.09 ± 2.64 and 6.06 ± 1.24 nM respectively; and AP3 showed the strongest antitumor effect.

Cell cycle

For understanding of the effect on cell cycle, U937 cells were treated with the aforementioned compounds during 24 hours at concentrations close to their IC_{50}, and then analyzed by flow cytometry. As shown in (**Figure 2**), after the cells were treated with AP3 and paclitaxel, large amount of the cells was found in G2/M phase. However, in the CPT group two concentration-depending effects were observed: at 25 nM most cells were arrested in G2/M phase and at 50 nM the arrest was happening in S phase. Percentages of the cells in different cell cycle phases are shown in (**Table 2**). For 0.1 nM - 0.4 nM of AP3 and 3 nM - 12 nM of paclitaxel, percentage of the cells in G2/M phase went up from about 30% to more than 60%, and at the same time the number of cells in G0/G1 and S phases decreased. As CPT concentration uprose from 12.5 nM to 25 nM, proportion of the cells in G0/G1 phase declined from about 30% to 15%, and percentage of cells in G2/M phase went up from about 45% to 60%. However, when the dose of CPT reached 50 nM, about 60% of the cells were registered in S phase, meanwhile the portion of cells in G2/M phase reduced to about 30%.

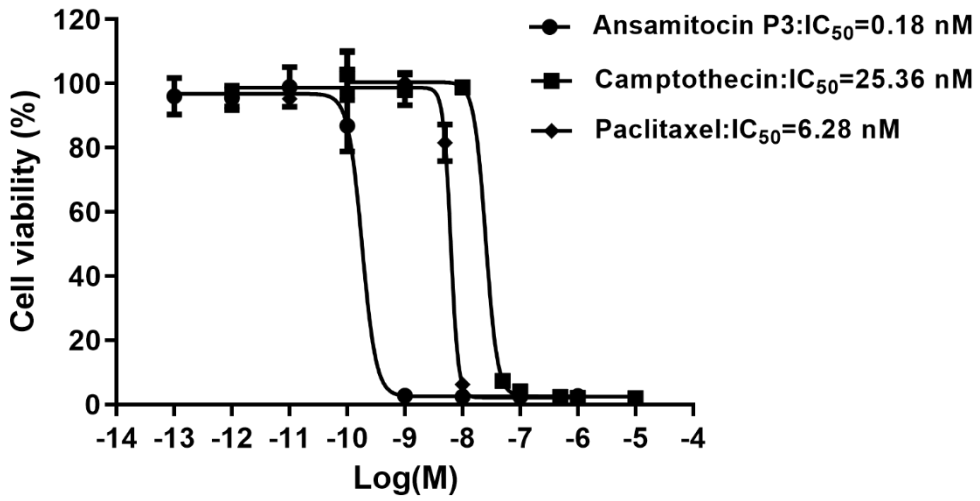

Figure 1 Viability of U937 cells after treatment with ansamitocin P3 (AP3), camptothecin (CPT) and paclitaxel for 72 hours. The concentrations for AP3 were set from 10^{-6} M to 10^{-13} M, for CPT - from 10^{-5} M to 10^{-10} M, and for paclitaxel - from 10^{-6} M to 10^{-12} M.

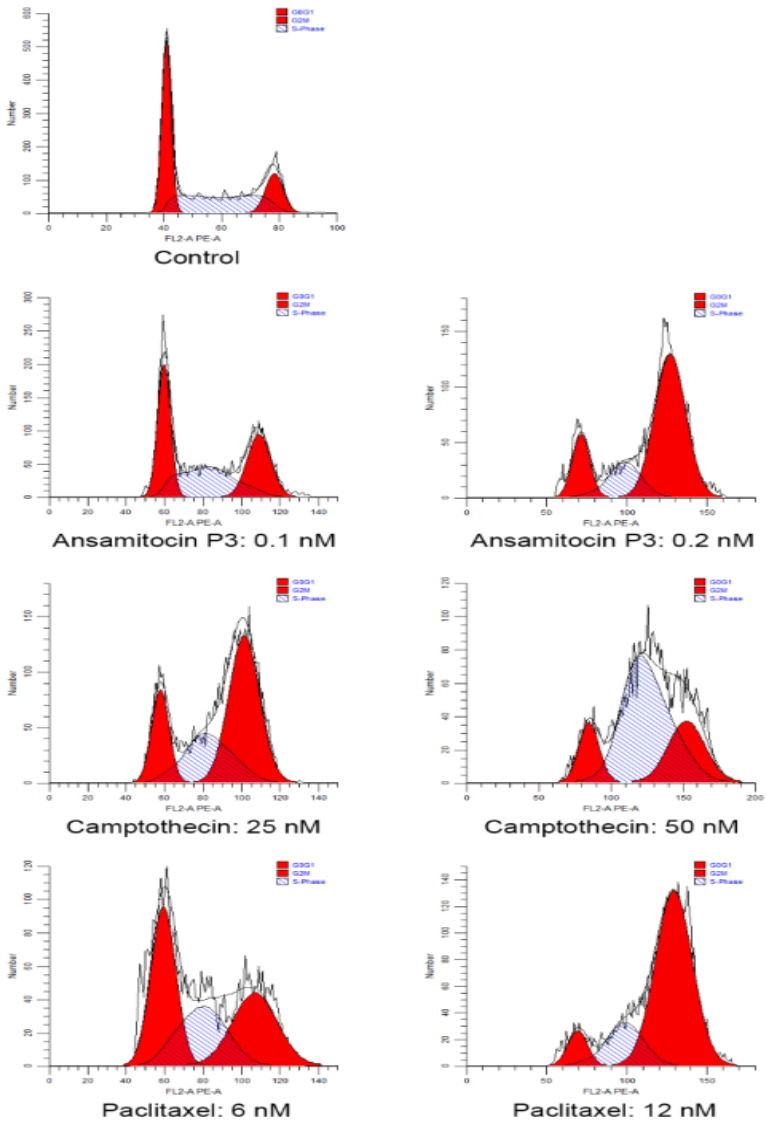

Figure 2 Cell cycle changes arrested by ansamitocin P3 (AP3), camptothecin (CPT) and paclitaxel. Data were analyzed by ModFit, and G0G1, G2M and S-Phase represent cells in G0/G1, G2/M and S phase, respectively. After treatment with high concentration of compounds for 24 hours, a great percentage of U937 cells was arrested in G2/M phase in AP3- or paclitaxel-treated group, but in S phase in CPT-treated group.

Table 2. Percentages of cells in different phases of cell cycle after treatment with the compounds for 24 hours.

	Concentration (nM)	G0/G1 phase (%)	S phase (%)	G2/M phase (%)
Control		44.82 ± 2.16	36.67 ± 2.33	18.52 ± 0.17
Ansamitocin P3	0.1	36.10 ± 1.53	34.06 ± 1.21	29.84 ± 0.33
	0.2	16.03 ± 0.66	15.58 ± 2.21	68.40 ± 2.86
	0.4	6.02 ± 2.91	18.62 ± 2.62	75.37 ± 5.53
Camptothecin	12.5	30.16 ± 3.34	35.60 ± 1.43	34.24 ± 1.91
	25	16.95 ± 3.22	27.83 ± 0.48	55.23 ± 2.74
	50	10.65 ± 3.42	70.82 ± 11.24	18.54 ± 7.81
Paclitaxel	3	40.56 ± 2.90	33.32 ± 8.81	26.12 ± 5.92
	6	32.70 ± 9.02	33.70 ± 7.09	33.61 ± 1.93
	12	5.59 ± 1.80	27.01 ± 12.30	66.41 ± 10.50

Cell apoptosis

The percentage of apoptotic U937 cells after treatment for 48 hours with each of the three compounds was measured by flow cytometry. As shown in (**Figure 3**), a number of both early (Q3)

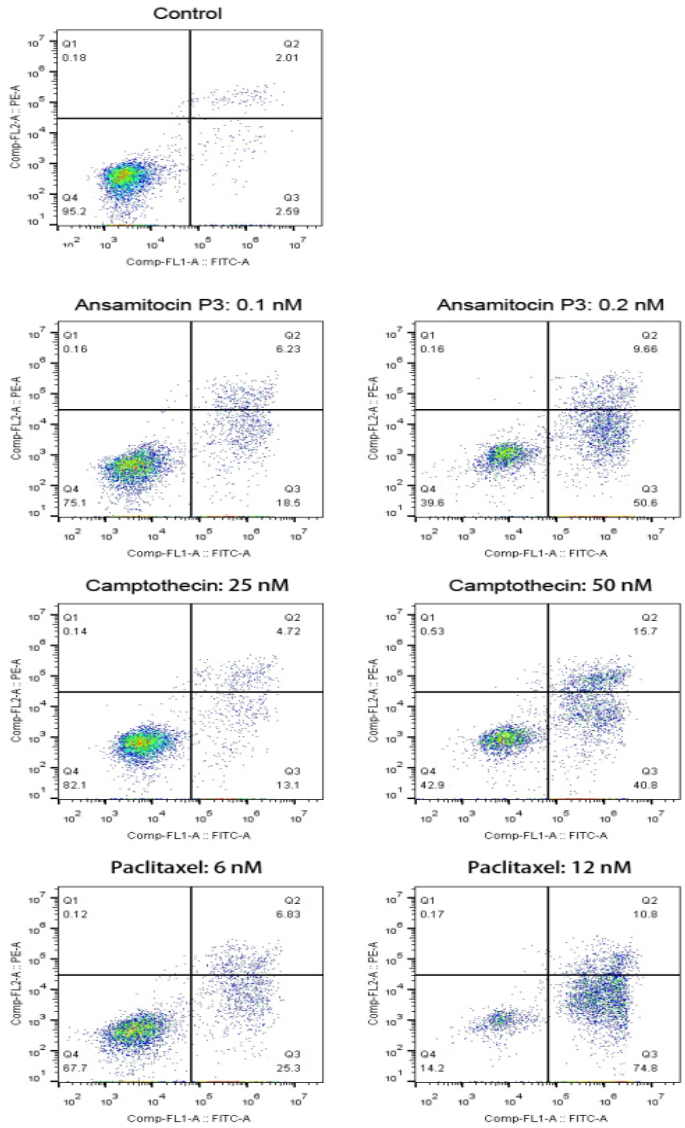

Figure 3 Cell apoptosis in histiocytic lymphoma U937 cells induced by ansamitocin P3, camptothecin and paclitaxel. Q1, Q2, Q3, Q4 represent dead, late apoptotic, early apoptotic and live cells, respectively. After treatment with the three compounds, both early and late apoptotic cells increased obviously.

and late (Q2) apoptotic cells was significantly increased in the treatment group, indicating that these compounds have great pro-apoptotic effect of on U937 cells. While in the control group the total percentage of the apoptotic cells was about 4.43 ± 0.25 %, in experimental groups treated with AP3 at concentrations ranging from 0.1 nM to 0.4 nM this value increased from 24.83 ± 0.13 % to 83.31 ± 0.47 %. With an increase in concentration of CPT from 12.5 nM to 50 nM and paclitaxel from 3 nM to 12 nM, the number of apoptotic cells raised from 5.54 ± 1.78 % to 54.65 ± 2.62 % for CPT and from 7.17 ± 0.20 % to 82.23 ± 4.77 % for paclitaxel (**Table 3**). These data demonstrates that the percentage of apoptotic cells was correlated with drug concentration, therefore all three compounds are cell apoptosis inducers.

Expression of proliferating cell nuclear antigen in U937 cells

To elucidate the putative mechanism of cell apoptosis we have chosen 0.2 nM concentrations for AP3, 50 nM for CPT and 12 nM for paclitaxel. U937 cells were treated for 24 hours with abovementioned doses. Then, the mRNA levels of BCL-2, P21, P63 and PCNA (proliferating cell nuclear antigen) were analyzed by qPCR. As shown in (**Figure 4**), BCL-2 was downregulated in all the groups treated with each of the three compounds, which is consistent with the high percentages of apoptotic cells. Besides, AP3 and paclitaxel treated groups ($P < 0.05$) demonstrated decreased expression of PCNA mRNA compared with the control group, but we did not observe significant difference in CPT-treated group.

Table 3. Percentages of apoptotic cells in U937 after treatment with the compounds.

	Concentration (nM)	Early apoptotic cells (%)	Late apoptotic cells (%)	Total Apoptotic cells (%)
Control		2.55 ± 0.06	1.88 ± 0.19	4.43 ± 0.25
Ansamitocin P3	0.1	18.55 ± 0.07	6.28 ± 0.06	24.83 ± 0.13
	0.2	54.90 ± 6.08	10.23 ± 0.81	65.13 ± 6.89
	0.4	74.10 ± 0.85	9.21 ± 0.38	83.31 ± 0.47
Camptothecin	12.5	3.43 ± 1.13	2.11 ± 0.65	5.54 ± 1.78
	25	12.10 ± 1.41	5.04 ± 0.45	17.14 ± 0.97
	50	37.80 ± 4.24	16.85 ± 1.63	54.65 ± 2.62
Paclitaxel	3	4.80 ± 0.64	2.38 ± 0.45	7.17 ± 0.20
	6	27.35 ± 2.90	7.51 ± 0.96	34.86 ± 3.85
	12	73.10 ± 2.40	9.13 ± 2.37	82.23 ± 4.77

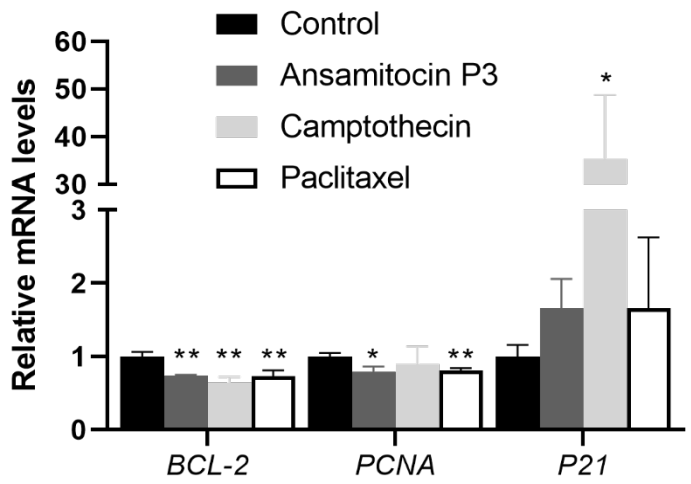

Figure 4 mRNA levels of BCL-2, P21 and PCNA in U937 cells subjected to 24 hours exposure to 0.2 nM AP3, 50 nM CPT and 12 nM paclitaxel. BCL-2 expression was downregulated in three groups. PCNA expression was downregulated in AP3 and paclitaxel treated groups. P21 expression was downregulated in CPT treated group. Statistical significance was determined by the t-test (* represents $p < 0.05$ and ** represents $p < 0.01$).

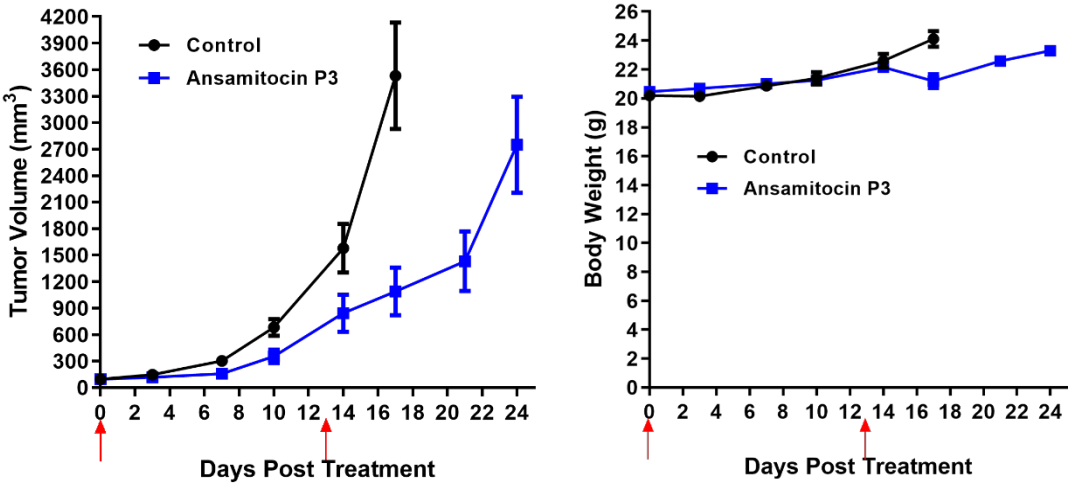

Figure 5 AP3 suppressive effect on U937-derived tumor growth in nude mice. AP3 (0.4 mg/kg, 100 µl) was administered twice by tail vein injection with an interval of two weeks, control group received injections with PBS. Data are presented as the mean ± S.E.M.

To explain the effect of cell cycle arrest, we examined the mRNA expression of P21. It was significantly upregulated after CPT treatment but showed only slight upregulation in AP3 and paclitaxel treated groups with no significant difference compared to the control group. In addition, the P63 mRNA expression was barely detectable in all groups (data not shown).

AP3 inhibited the in vivo growth of U937-derived xenograft tumor.

To investigate the antitumor efficacy of AP3 in vivo, U937-derived **Mouse Xenograft Tumor Models** were established. When the size of tumors reached 70-120 mm^3, the mice were divided into two groups (n=8 each): AP3 treatment group, which received 100 µl tail vein injection with the dose of 0.4 mg/kg, and the control, which received the PBS. As shown in (Figure. 5), tumor volume in the control group uprose from 94.96 ± 12.03 mm^3 (Day 0) to 3530.20 ± 1595.66 mm^3 (Day 17). Mice of the control group were sacrificed at day 17 according to the requirement of the ethics of animal experiments. In AP3 treatment group, tumor volumes went up from 95.28 ± 9.98 mm^3 (Day 0) to 2749.61 ± 1442.53 mm^3 (Day 24). The relative tumor proliferation rate (T/C, %) was 29.98 at Day 17, and bodyweight decrease was insignificant. The results support that treatment with a low dose of 0.4 mg/kg of AP3 once per two weeks inhibits U937-derived xenograft tumor growth.

Discussion

Ansamitocin P3 (AP3), paclitaxel and camptothecin (CPT) are highly effective antitumor biomolecules. We were the first group to have compared the cytotoxic effect of AP3, paclitaxel and CPT on U937 lymphoma cells in vitro. No more than 10% of cells survived after treatment with these three compounds, and AP3 showed the strongest anti-tumor effect with the lowest IC_{50}.

Cell cycle analysis demonstrated that a lot of cells were arrested in G2/M phase after treatment with increasing concentration of AP3 and paclitaxel tubulin inhibitors; these data are consistent with the previous data showing the ability of these compounds to arrest U937 cells in mitosis [8, 12]. We found that cells are arrested in G2/M phase when treated with low concentration of CPT but in S phase in case of high concentration; and these observations are related to previously reported Topo I activity [31]. Low concentration of CPT might cause low levels of DNA damage which leads to cells accumulating at G2 check point [32]. When treated with high concentration of CPT, cells suffer severe DNA damage and thus are arrested in S phase.

Apoptosis analysis data showed treatment with all three compounds for 48 hours resulted in increased percentages of both early and late apoptotic cells which positively correlates with drug concentration. More than half of the cells underwent apoptosis at high concentration of compounds suggesting the strong antitumor effect of the aforementioned biomolecules.

To explore the difference in mechanisms by which the compounds exhibit their inhibitory effects, expressions of several genes were analyzed by qPCR. We found that BCL-2, which is believed to be a common anti-apoptotic protein [33-35], is downregulated in cells treated with each of the three compounds. We have also analyzed the expression of proliferating cell nuclear antigen (PCNA), which is known to facilitate DNA replication and repair and to be involved in apoptosis signaling pathway [36]. It was reported earlier that paclitaxel inhibits PCNA in MG-63 cells [37], and our study confirmed the similar effect on U937 cells. Indeed, PCNA was downregulated after treatment with both AP3 and paclitaxel, suggesting their role in proliferation inhibition. However, no difference was observed between the CPT-treated and the control group. Known cyclin-dependent kinase (CDK) inhibitor and apoptosis inhibitor [38, 39] P21 was essentially upregulated in CPT-treated group when compared to the control group, which is consistent with the earlier data [40], but only slightly upregulated in AP3- or paclitaxel-treated group without any statistically significant difference. There results indicate that though CPT, AP3 and paclitaxel use the same signaling pathway, there is a difference in the cell apoptosis induced by each of them.

In the xenograft animal model, tumor volumes in AP3 treatment group of mice were obviously smaller than that in the control group. At day 17, the relative tumor proliferation rate (T/C, %) was close to 30, indicating the great antitumor effect of AP3. At the same time, treatment scheme of two injections with an interval of two weeks between them resulted in a slight decrease in bodyweight.

To summarize, we compared the effect of AP3, paclitaxel and CPT on inhibition of U937 lymphoma cells in vitro and confirmed that AP3 has the same mechanism in inducing apoptosis as paclitaxel, but with stronger antitumor activity. The in vivo anti-tumor assay also showed that AP3 greatly inhibits tumor growth with mild side effects. Development of biomolecule conjugation technology and tumor targeting therapy capable to selectively kill tumor cells requires the selection of agents with high cytotoxicity. Our results suggest that AP3 shows great potential in treatment of lymphoma and thus can be considered a suitable candidate biomolecule for tumor targeting therapy.

Acknowledgement

We would like to acknowledge the great support given to us by Shenzhen Science and Technology Program (Grant No: KQTD20170810154011370), Xiangtan Institute of Industrial Technology Collaborative Innovation, and Xiangtan Science and Technology.

References

1 Armitage JO, Gascoyne RD, Lunning MA, Cavalli F (2017) Non-hodgkin lymphoma. Lancet 390: 298-310.

2 Freedman AS, LaCasce AS (2016) Non-Hodgkin's Lymphoma. Holland Frei Cancer Medicine 1-19.

3 Wang L, Qin W, Huo Y-J, Li X, Shi Q, et al. (2020) Advances in targeted therapy for malignant lymphoma. Signal Transduct Tar 5: 1-46.

4 Khongorzul P, Ling CJ, Khan FU, Ihsan AU, Zhang J (2020) Antibody–drug conjugates: a comprehensive review. Mol Cancer Res 18: 3-19.

5 Yaghoubi S, Karimi MH, Lotfinia M, Gharibi T, MahiBirjand M, et al. (2020) Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy. J Cell Physiol 235: 31-64.

6 Gezici S, Sekeroğlu N (2019) Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents. Anti-Cancer Agent Med Chem 19: 101-111.

7 Chen L, Zhang Q-Y, Jia M, Ming Q-L, Yue W, et al. (2016) Endophytic fungi with antitumor activities: Their occurrence and anticancer compounds. Crit Rev Microbiol 42: 454-473.

8 Steinmetz MO, Prota AE (2018) Microtubule-targeting agents: strategies to hijack the cytoskeleton. Trends Cell Biol 28: 776-792.

9 Mukhtar E, Adhami VM, Mukhtar H (2014) Targeting microtubules by natural agents for cancer therapy. Mol Cancer Ther 13: 275-284.

10 Lemjabbar-Alaoui H, Peto CJ, Yang Y-W, Jablons DM (2020) AMXI-5001, a novel dual parp1/2 and microtubule polymerization inhibitor for the treatment of human cancers. Am J Cancer Res 10: 2649-2676.

11 Higashide E, Asai M, Ootsu K, Tanida S, Kozai Y, et al. (1977) Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from Nocardia. Nature 270: 721-722.

12 Venghateri JB, Gupta TK, Verma PJ, Kunwar A, Panda D (2013) Ansamitocin P3 depolymerizes microtubules and induces apoptosis by binding to tubulin at the vinblastine site. PloS one.

13 Cassady J M, Chan K K, Floss H G, Leistner E (2004) Recent developments in the maytansinoid antitumor agents. Chem Pharm Bull 52: 1-26.

14 McGuire WP, Rowinsky EK, Rosenshein NB, Grumbine FC, Ettinger DS, et al. (1989) Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann Intern Med 111: 273-279.

15 Marupudi NI, Han JE, Li KW, Renard VM, Tyler BM, et al. (2007) Paclitaxel: a review of adverse toxicities and novel delivery strategies. Expert Opin Drug Saf 6: 609-621.

16 Markman M, Mekhail TM (2002) Paclitaxel in cancer therapy. Expert Opin Pharmacol 3: 755-766.

17 Alves RC, Fernandes RP, Eloy JO, Salgado HRN, Chorilli M (2018) Characteristics, properties and analytical methods of paclitaxel: a review. Crit Rev Anal Chem 48: 110-118.

18 Alqahtani FY, Aleanizy FS, Tahir E, Alkahtani HM, AlQuadeib BT (2019) Chapter Three—Paclitaxel.Profiles Drug Subst Excip Relat Methodol 44: 205-238.

19 Wang TH, Wang HS, Soong YK (2000) Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. Cancer 88: 2619-28.

20 Brito DA, Yang Z, Rieder CL (2008) Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. J Cell Biol 182: 623-629.

21 Bernabeu E, Cagel M, Lagomarsino E, Moretton M, Chiappetta DA (2017) Paclitaxel: what has been done and the challenges remain ahead. Int J Pharmaceut 526: 474-495.

22 Adrianzen Herrera D, Ashai N, Perez-Soler R, Cheng H (2019) Nanoparticle albumin bound-paclitaxel for treatment of advanced non-small cell lung cancer: an evaluation of the clinical evidence. Expert Opin Pharmacol 20: 95-102.

23 Martino E, Della Volpe S, Terribile E, Benetti E, Sakaj M, et al. (2017) The long story of camptothecin: From traditional medicine to drugs. Bioorg Med Chem Lett 27: 701-707.

24 Venditto VJ, Simanek EE (2010) Cancer therapies utilizing the camptothecins: a review of the in vivo literature. Mol Pharmaceut 7: 307-349.

25 Li F, Jiang T, Li Q, Ling X (2017) Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer? Am J Cancer Res 7: 2350.

26 Liu LF, Desai SD, Li TK, Mao Y, Sun M, et al. (2000) Mechanism of action of camptothecin. Ann Ny Acad Sci 922: 1-10.

27 Bailly C (2019) Irinotecan: 25 years of cancer treatment. Pharmacol Res.

28 Kciuk M, Marciniak B, Kontek R (2020) Irinotecan—Still an important player in cancer chemotherapy: A comprehensive overview. Int J Mol Sci 21: 4919.

29 Moertel CG, Schutt AJ, Reitemeier R, Hahn R (1972) Phase IT study of camptothecin (NSC-1 00880) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 56: 95-101.

30 García-Alonso S, Ocaña A, Pandiella A (2020) Trastuzumab emtansine: mechanisms of action and resistance, clinical progress, and beyond. Trends Cancer 6: 130-146.

31 Jones CB, Clements MK, Wasi S, Daoud SS (1997) Sensitivity to camptothecin of human breast carcinoma and normal endothelial cells. Cancer Chemoth Pharm 40: 475-483.

32 Goldwasser F, Shimizu T, Jackman J, Hoki Y, O'Connor PM, et al. (1996) Correlations between S and G2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. Cancer Res 56: 4430-4437.

33 Singh R, Letai A, Sarosiek K (2019) Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Bio 20: 175-193.

34 Ashkenazi A, Fairbrother WJ, Leverson JD, Souers AJ (2017) From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nat Rev Drug Discov 16: 273-284.

35 Marquez RT, Liang X (2012) Bcl-2: Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch. Am J Cancer Res 2: 214-221.

36 González-Magaña A, Blanco FJ (2020) Human PCNA structure, function and interactions. Biomolecules.

37 Liu S-Y, Song S-X, Lin L, Liu X (2010) Molecular mechanism of cell apoptosis by paclitaxel and pirarubicin in a human osteosarcoma cell line. Chemotherapy 56: 101-107.

38 Shamloo B, Usluer S (2019) p21 in cancer research. Cancers.

39 Karimian A, Ahmadi Y, Yousefi B (2016) Multiple functions of p21

in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA repair 42: 63-71.

40 Chiu C-F, Lin Y-Q, Park J M, Chen Y-C, Hung S-W, et al. (2020) The novel camptothecin derivative, CPT211, induces cell cycle arrest and apoptosis in models of human breast cancer. Biomed Pharmacother.