A THESIS FOR THE DEGREE OF MASTER

Anti-Tumour Effects of Paclitaxel on Canine Osteosarcoma and Melanoma Cell Lines

개의 골육종 및 흑색종 세포주에서의 파클리탁셀의 항암 효과

2018년 2월

서울대학교 대학원

수의과대학 수의내과학 전공

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지도교수 윤화영

이 논문을 수의학석사학위논문으로 제출함.

2017년 12월

서울대학교 대학원

수의과대학 수의내과학 전공

김 수 연

김수연의 석사학위논문을 인준함.

2017년 12월

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Anti-Tumour Effects of Paclitaxel

on Canine Osteosarcoma and Melanoma Cell Lines

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Abstract

In dogs, solid tumours are one of the most life-threatening diseases, including mammary gland tumours, mast cell tumours, squamous cell carcinoma, osteosarcoma, and malignant melanoma. Paclitaxel, a chemotherapeutic agent from the taxane family, is used for the treatment of human and canine solid tumours. A new oral formulation of paclitaxel (Liporaxel® Sol.) has been developed, but its anti-tumour effects on malignant canine tumour are unknown. The purpose of this study was to determine the anti-tumour effects of paclitaxel on canine osteosarcoma and melanoma cell lines and to assess the clinical safety of oral paclitaxel in normal dogs.

The anti-tumour effects of paclitaxel on D17 (canine osteosarcoma cells) and LMeC (canine melanoma cells) were detected by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay, cell cycle analysis, and annexin-V assay. To evaluate the safety of oral paclitaxel, three healthy dogs were orally administered 5 mg/kg paclitaxel every 7 or 14 days for 8 weeks. Complete blood counts and serum chemistry analyses were performed and the data were compared by one-way analysis of variance.

Paclitaxel significantly inhibited the proliferation of D17 and LMeC cells. The flow cytometric analysis revealed that the cell cycles in both malignant cell lines were arrested in the G2/M phase and that paclitaxel induced cell apoptosis. The healthy dogs that received oral paclitaxel for 8 weeks did not exhibit any clinical signs and all blood parameters were within the normal ranges.

In conclusion, oral paclitaxel might be a viable, novel chemotherapeutic agent for malignant canine tumours, particularly osteosarcoma and melanoma.

Key words: oral paclitaxel, liporaxel, antitumour agent, canine osteosarcoma, canine melanoma

Student number: 2015-23158

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1. Introduction

In dogs, solid tumours are one of the most life-threatening diseases. The most common types of solid tumours are mammary gland tumours, mast cell tumours, squamous cell carcinoma, osteosarcoma, and malignant melanoma. Osteosarcoma is the most common primary bone tumour in dogs and constitutes up to 85% of malignancies that originate in the skeleton (Ling et al. 1974; Liu et al. 1977). Melanoma is the most common oral malignancy in dogs with significant levels of skin pigmentation (Smith et al. 2002; Todoroff and Brodey 1979; Wallace et al. 1992). The primary treatment for both osteosarcoma and melanoma is surgery. However, for late-stage disease or one that is highly metastasized, chemotherapy is recommended.

Paclitaxel is an anti-tumour chemotherapeutic agent from the taxane family widely used in the treatment of human solid tumours. It is extracted from the bark of the Pacific yew tree, *Taxus brevifolia* (Poirier et al. 2004). In veterinary medicine, paclitaxel is also used for the treatment of various types of solid tumours via intravenous (IV) infusion or subcutaneous (SC) administration. A previous clinical study demonstrated that IV and SC paclitaxel exerted significant anti-tumour effects in canine cancer patients (Silva et al. 2015; von Euler et al. 2013).

However, IV paclitaxel infusions have shown acute and severe hypersensitivity, because paclitaxel is insoluble in aqueous solution and must be combined with polyethoxylated castor oil (cremophor-EL) (Moon et al. 2000; Poirier et al. 2004). Owing to these allergic reactions, pre-medication with antihistamine agents and corticosteroids is necessary (Kloover et al. 2004). Although the SC administration of paclitaxel has a lower risk of hypersensitivity than an IV infusion, it causes local inflammatory reactions and stresses generally associated with

repeated needle puncture. Compared with the IV or SC administration of paclitaxel, oral paclitaxel is practical and convenient for dogs, because it is only administered once per week and does not require the administration of a pre-medication.

The aim of this study was to investigate the efficacy of paclitaxel on canine osteosarcoma and melanoma cell lines and the safety of orally administered paclitaxel in normal healthy dogs.

2. Materials and Methods

2.1. Cell culture

The canine osteosarcoma cell line D17 (ATCC CCL-183) was kindly provided by the Department of Veterinary Pharmacology, Seoul National University. The cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (H-DMEM; PAN Biotech, Aidenbach, Germany) supplemented with 10% foetal bovine serum (FBS; PAN Biotech), 1% 100 units/mL penicillin, and 100 μg/mL streptomycin (P/S; PAN Biotech) at 37°C in a humidified atmosphere containing 5% CO_2. The canine melanoma cell line LMeC (Inoue et al., 2004) was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (PAN Biotech) supplemented with 10% FBS and 1% P/S. The cells were incubated in 5% CO_2 at 37°C and the media was replenished every 3 days. When the cells reached 90% confluence, they were expanded in new media. The cells were used between passages 10 to 15.

2.2. Isolation of canine peripheral blood mononuclear cells (cPBMC)

Blood samples were collected from a normal healthy dog kept at the Veterinary Medical Teaching Hospital of Seoul National University (SNU VMTH), mixed with an equal volume of phosphate-buffered saline (PBS), and layered over Ficoll-Paque PLUS (GE Healthcare Life Sciences, Uppsala, Sweden) in conical tubes. After centrifugation at 750 × *g* for 30 min, the cell layer was collected. RBC lysis buffer was added and the cells were washed with PBS.

cPBMCs were resuspended in RPMI-1640 medium (PAN Biotech) supplemented with 10% FBS. PBMCs were inoculated in a 96-well plate at a concentration of 1×10^5 cells/well and some of PBMCs were stimulated with 25 μg/mL concanavalin A (ConA).

2.3. Anti-tumour agents

Oral paclitaxel solution (Liporaxel soln.) was offered by Daehwa Pharmaceutical Co. Ltd (Hoengseong-gun, Gangwon-do, Korea). The solution was stored at 4°C and administered at doses of 0, 1.25, 2.5, and 5 μg/mL in dimethyl sulphoxide (DMSO) for the in vitro experiments, in accordance with previous studies (Seo et al. 2011). For the in vivo experiments, normal healthy dogs from SNU VMTH were administered the drug at 5 mg/kg.

2.4. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The proliferation of cells was evaluated by MTT assay. D17 and LMeC cells were inoculated in a 96-well plate at 2,000 cells/well and incubated for 24, 48, and 72 h in the absence or presence of 0–5 μg/mL paclitaxel. Subsequently, 20 μL 5 mg/mL MTT solution was added and incubated in the dark at 37°C for 2 h. After the removal of the MTT solution, the reaction was terminated by the addition of 100 μL DMSO. The absorbance at 540 nm, with a correction at 650 nm, was determined by a spectrophotometer (680 Microplate Reader, Bio rad, Milton-Freewater, Oregon, USA).

2.5. Cell cycle analysis

For flow cytometry (FACS) analysis, 2×10^5 cells/well were seeded into 6-well plates and left to adhere overnight at 37°C in an atmosphere of 5% CO_2. The cells were incubated with 0, 1.25, and 2.5 μg/mL paclitaxel and incubated again 24 h. After incubation, both floating and attached cells were harvested, washed with PBS, re-suspended, and fixed in 70% ethanol at 4°C. The cells were then incubated with 500 μL propidium iodide (PI)/RNase buffer (BD Biosciences, San Diego, CA, USA) for 30 min at room temperature. The samples were analysed with flow cytometry (FACS AriaII Automated High-speed Flow Cytometry System, San Diego, CA, USA).

2.6. Apoptosis detection with flow cytometry

The annexin V-FITC staining was used to distinguish living cells and apoptotic cells. The D17 and LMeC cell lines were treated with 0, 1.25, and 2.5 μg/mL paclitaxel, cultured in an atmosphere of 5% CO_2 at 37°C for 24 h, harvested, washed with PBS, and then resuspended in PBS. The cells were stained with annexin V-FITC in accordance with to the protocol of Annexin V-FITC cell Apoptosis Detection Kit (Enzo, Farmingdale, NY, USA). The absorbance of samples was measured by flow cytometry (FACS AriaII Automated High-speed Flow Cytometry System).

2.7. Animal experiments

The study and all experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-170704-13), and the animal study protocol was performed in accordance with the approved guidelines.

Three normal healthy 2-4-year old beagle dogs were housed under controlled conditions of temperature, humidity and light cycle. They were orally administered 5 mg/kg paclitaxel every 7 days for 3 weeks. After 3 weeks of administration, 1 week was rest period. After the rest period, three healthy dogs were orally administered paclitaxel again every 7 days for 3 weeks. Viability, appetite and vomiting signs were observed. Blood samples were obtained before starting administration and after every administration. Complete blood counts (white blood cell count, red blood cell count, haematocrit, platelet count), serum chemistry analyses (Alanine transaminase, Aspartate aminotransferase, Alkaline phosphatase, Gamma-glutamyl transferase, Bilirubin, Glucose, Total protein, Albumin, Blood urea nitrogen, Creatinine, Calcium, Phosphate) and electrolytes were performed.

2.8. Statistical analysis

All the experimental data were analysed by using the GraphPad Prism v.6 software (Graphpad Software Inc., San Diego, CA, USA). All data have been expressed as the mean ± standard deviation (SD). Statistical analysis was performed by using one-way ANOVA, with differences of $P < 0.05$ considered statistically significant.

3. Results

3.1. MTT assay for D17 and LMeC cells

The MTT assay indicated that paclitaxel treatment significantly inhibited the viability of D17 and LMeC cells. In comparison with the negative controls, D17 cells treated with 2.5 μg/mL paclitaxel showed decreased viability of 31.38% at 48 h and 14.63% at 72 h (Fig. 1A); similarly, LMeC cells treated with 2.5 μg/mL paclitaxel showed decreased viability of 33.85% at 48 h and 14.97% at 72 h (Fig. 1B). The viability of D17 and LMeC cells treated with paclitaxel decreased with an increasing concentration of the drugs at 24 h. At 48 h and 72 h, the viability of cells treated with 1.25, 2.5, or 5 μg/mL paclitaxel resulted in no significant differences.

3.2. Cell cycle analysis

The effects of paclitaxel on cell cycle progression in D17 and LMec cell lines were investigated by flow cytometry. The cells were incubated with different concentrations (0, 1.25, and 2.5 μg/mL) of paclitaxel for 24 h and were analysed for the distribution of sub-G1, G0/G1, S, and G2/M phases of cell cycle. The normal D17 and LMeC cell populations were the highest in the G0/G1 phase (P3) and the lowest in the G2/M phase (P5). In comparison with the control, paclitaxel treatment resulted in a significant accumulation of cells in the G2/M phase, which was accompanied by a decrease in the G0/G1 phase in D17 cells and the

P3/P5 ratio decreased (Fig. 2A). Similar to that in D17 cells, the P3/P5 ratio in the LMeC cycle was significantly decreased in comparison with the control (Fig. 2B). The P3/P5 ratio in D17 and LMeC cycle after treatment with 2.5 μg/mL showed a greater decrease than that after treatment with 1.25 μg/mL (Fig. 2C, 2D); however, the difference was not significant.

3.3. Annexin-V for evaluation of cell damage

D17 and LMeC cells were incubated with different concentrations (0, 1.25, and 2.5 μg/mL) of paclitaxel for 24 h, stained with annexin V, and analysed by flow cytometry. D17 cells treated with 1.25 and 2.5 μg/mL paclitaxel resulted in 22.0 and 26.8% apoptosis, respectively (Fig. 3A). Similar to D17 cells, LMeC cells treated with 1.25 and 2.5 μg/mL paclitaxel resulted in 18.0 and 22.8% apoptosis (Fig. 3B). The apoptosis rate of D17 and LMeC increased with an increase in paclitaxel concentrations. The apoptosis rate in D17 cells treated with 2.5 μg/mL paclitaxel was three times higher than that of the negative control (Fig. 3C). In addition, the apoptosis rate in LMeC cells treated with 2.5 μg/mL paclitaxel was two times higher than that of the negative control (Fig. 3D).

3.4. MTT assay for PBMCs

To determine the safety of paclitaxel against normal cells, the viability of peripheral blood mononuclear cells (PBMCs) treated with different concentrations (0, 1.25, and 2.5 μg/mL) of paclitaxel was evaluated. The absorbance of paclitaxel-treated PBMCs incubated for 24, 48, and 72 h was not significantly different from that of the negative control (Fig. 4A). ConA was added to stimulate PBMCs. The results showed that the viability of stimulated PBMCs was

not affected by paclitaxel treatment at concentrations up to 2.5 μg/mL for up to 72 h (Fig. 4B).

3.5. Clinical safety of oral paclitaxel

Viability, appetite and vomiting signs were observed. There was no change in healthy dogs (Table 1). The haematological toxicity was evaluated through the measurement of neutropenia, anaemia, and thrombocytopenia (Table 2). Serum chemistry was measured to evaluate the side effects of oral paclitaxel on the liver, kidney, and electrolytes (Table 3). The results indicated no obvious side effects in normal dogs that were orally administered 5 mg/kg paclitaxel every 7 days for 6 weeks.

4. Discussion

In this study, I proved the efficacy of paclitaxel in the canine malignant cell lines, D17 and LMeC, and confirmed the safety of oral paclitaxel in normal healthy dogs, which comprised an initial step for the consideration of oral paclitaxel as an anti-tumour agent for veterinary cancers.

The MTT assay demonstrated that paclitaxel significantly inhibited D17 and LMeC cell proliferation and viability. The viability of D17 and LMeC treated with paclitaxel was suppressed in a concentration-dependent manner after paclitaxel incubation for 24 h. After 48 and 72 h of paclitaxel incubation, the viability of D17 and LMeC cells treated with the lowest dose of paclitaxel was significantly inhibited, but no concentration-dependent inhibition was observed. A significant inhibitory effect on the proliferation of D17 and LMeC cells was observed in a time-dependent manner. For normal canine PBMCs, no obvious inhibitory effect was observed after equal doses of paclitaxel incubation for 24, 48, and 72 h. Not only naïve cPBMC, but also cPBMC stimulated by ConA, showed a normal level of proliferation after incubation with paclitaxel for 24, 48, and 72 h. These data may suggest that paclitaxel has a selective capability to inhibit the malignant canine cell lines; normal cells and proliferating cells stimulated by ConA were unaffected by paclitaxel treatment. Recent studies have demonstrated that canine mesenchymal stromal cells loaded with paclitaxel inhibited the proliferation of a canine glioma cell line and two human glioblastoma cell lines (Bonomi et al. 2017). I observed similar results in D17 and LMeC cells.

Tumorigenesis results in uncontrolled cell cycle regulation (Golias et al. 2004; Nakayama

and Nakayama 2006; Sandal 2002). In the control group, more cells were found in the P3 phase (G0/G1 phase) in the D17 and LMeC cell lines. However, when the cells were treated with a higher dose of paclitaxel, the number of cells in the P5 phase (G2/M phase) significantly increased. It is believed that paclitaxel inhibits cell growth via cell cycle arrest in the P5 phase (G2/M phase), immediately prior to mitosis.

Apoptosis is the major cause of cell death induced by anti-tumour agents (Brown and Attardi 2005; Brown and Wouters 1999; Wang and Lippard 2005). Annexin V flow cytometric assays were performed to determine the cell damage induced by paclitaxel in D17 and LMeC cells. The stained cells indicated dead cells, which were in the stage of apoptosis. The apoptosis rate significantly increased in a concentration-dependent manner after incubation of paclitaxel for 24 h in both D17 and LMeC cell lines. The D17 cell line was more sensitive than LMeC at a low concentration of paclitaxel.

These data suggested that paclitaxel exerted anti-tumour effects on the canine malignant cell lines, D17 and LMeC, in the in vitro experiment. However, in a previous study, the clinical use of paclitaxel via IV or SC administration for canine cancer patients was proven effective, but presented a risk of side-effects such as hypersensitivity, cellulitis, and local inflammatory reaction (Kim et al. 2001; Kim et al. 2004; Silva et al. 2015). The risks of paclitaxel could be reduced by another administration route, such as oral administration, which has been used in human cancer patients (Britten et al. 2000; Kruijtzer et al. 2003; Ly et al. 2009; Malingré et al. 2000). However, the safety and efficacy of oral paclitaxel were not known in dogs. This is the first study to evaluate the safety of the oral administration of paclitaxel to dogs. The initial dosage was selected on the basis of the results of previous studies by Daehwa Pharmaceutical Co. Ltd (Hong et al. 2007). After administration, the results of the physical examination were normal in dogs. The results of the blood test were

similar to those of animals in the normal group.

In conclusion, my study demonstrated that paclitaxel exerted anti-proliferative effects on D17 and LMeC cells through the induction of G2/M phase cell cycle arrest and apoptosis. Moreover, paclitaxel did not exert this effect in normal canine cells. These results contribute to our understanding of the mechanism of action of paclitaxel in canine osteosarcoma and melanoma cells. There were no significant side effects in normal healthy dogs that received the therapeutic dose of oral paclitaxel. These results suggested that oral paclitaxel could be a realistic clinical anti-tumour agent for canine cancer patients, especially for osteosarcoma and melanoma. Further studies are necessary to investigate the clinical efficacy of oral paclitaxel on canine cancer patient.

Table 1. Complete blood cell counts in experimental dogs that received oral paclitaxel.

Dogs	Items	Reference range	Pre (0 day)	1st (7 days)	2nd (14 days)	3rd (21 days)	4th (35 days)	5th (42 days)	6th (49 days)
Beagle 1	WBC	5.2–17.0 (10^3/μL)	7.57	9.39	6.68	9.71	9.65	8.52	9.30
	RBC	5.70–8.80 (10^6/μL)	6.60	6.70	6.36	6.25	6.98	6.32	6.49
	Haematocrit	37.1–57.0 (%)	44.5	45.7	43.7	42.6	47.6	42.6	44.2
	Platelet	143–400 (10^3/μL)	204	220	241	205	202	199	203
Beagle 2	WBC	5.2–17.0 (10^3/μL)	6.60	9.54	9.80	9.17	11.55	8.97	6.63
	RBC	5.70–8.80 (10^6/μL)	8.24	6.69	7.17	7.13	7.57	7.06	8.03
	Haematocrit	37.1–57.0 (%)	55.2	47.3	50.6	50.8	53.7	49.8	53.2
	Platelet	143–400 (10^3/μL)	260	337	377	373	439	370	240
Beagle 3	WBC	5.2–17.0 (10^3/μL)	9.20	6.90	5.19	7.00	6.87	7.36	11.9
	RBC	5.70–8.80 (10^6/μL)	6.89	7.83	7.95	7.74	8.17	8.42	7.26
	Haematocrit	37.1–57.0 (%)	48.0	52.7	53.9	52.4	54.3	56.2	51.4
	Platelet	143–400 (10^3/μL)	438	293	319	262	255	281	376

Table 2. Serum chemistry of experimental dogs that received oral paclitaxel.

		Beagle 1			Beagle 2			Beagle 3		
	Reference range	Pre (0 day)	1 cycle (21 days)	2 cycles (49 days)	Pre (0 day)	1 cycle (21 days)	2 cycles (49 days)	Pre (0 day)	1 cycle (21 days)	2 cycles (49 days)
Na	145.1-(mmol/L)	142.5	140.8	140.1	142.8	140.8	144.8	146.3	145.0	147.6
K	3.6-5.5(mmol/L)	4.33	4.79	4.49	4.26	4.98	4.65	4.53	4.93	4.26
Cl	113.2-(mmol/L)	114.7	115.5	115.3	115.3	117.2	118.2	116.4	119.4	117.0
AST	11-42(U/L)	29	28	27	17	24	15	30	16	33
ALT	5-83(U/L)	41	35	36	37	43	45	40	45	49
ALP	0-97.9(U/L)	18	13	13	28	26	23	29	22	47
GGT	0-14(mg/dl)	5	4	3	5	5	4	4	2	7
Total bilirubin	0-0.2(mg/dl)	0.02	0.02	0.01	0.06	0.08	0.02	0.06	0.04	0.03
Glucose	74-120(mg/dl)	99	102	88	86	94	83	100	81	94
Total protein	5.7-7.5(g/dl)	6.79	6.11	6.58	7.12	7.12	6.5	7.56	6.73	7.53
Albumin	2.6-4.4(g/dl)	3.94	3.56	3.76	4.06	3.66	3.81	3.89	4.01	3.85
BUN	9-31.4(mg/dl)	10.1	15.0	11.2	11.6	12.5	11.0	16.3	15.7	19.3
Creatinine	0.4-1.3(mg/dl)	0.65	0.84	0.84	0.67	0.59	0.84	0.60	0.66	0.72
Calcium	9-11.9(mg/dl)	11.3	10.5	10.9	11.8	10.8	10.8	11.3	10.7	11.2
Phosphorous	1.3-6.3(mg/dl)	4.9	4.6	5.0	4.3	4.3	4.9	4.4	4.5	4.8

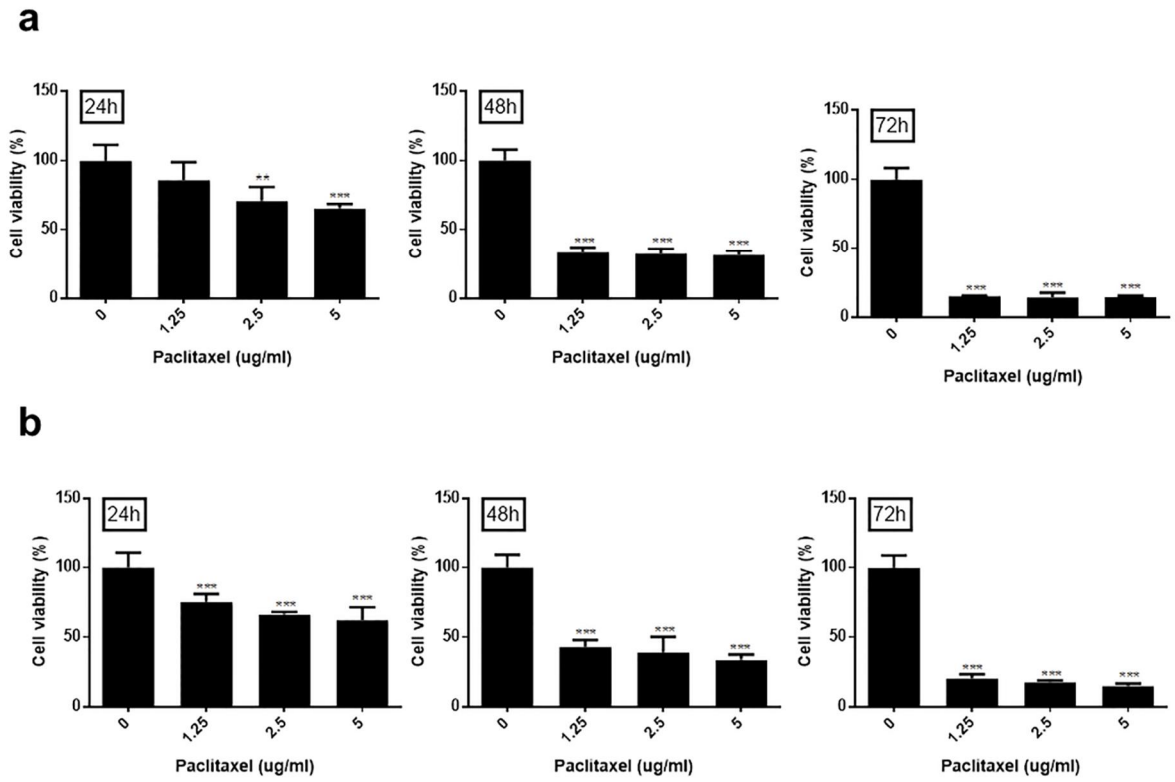

Figure 1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for D17 and LMeC cells treated with various concentrations of paclitaxel. (a) The viability of D17 cells treated with different concentrations of paclitaxel. (b) The viability of LMeC cells treated with different concentrations of paclitaxel. All experiments were independently conducted in triplicate. The data are presented as the mean ± standard deviation. ** $P < 0.01$, *** $P < 0.001$.

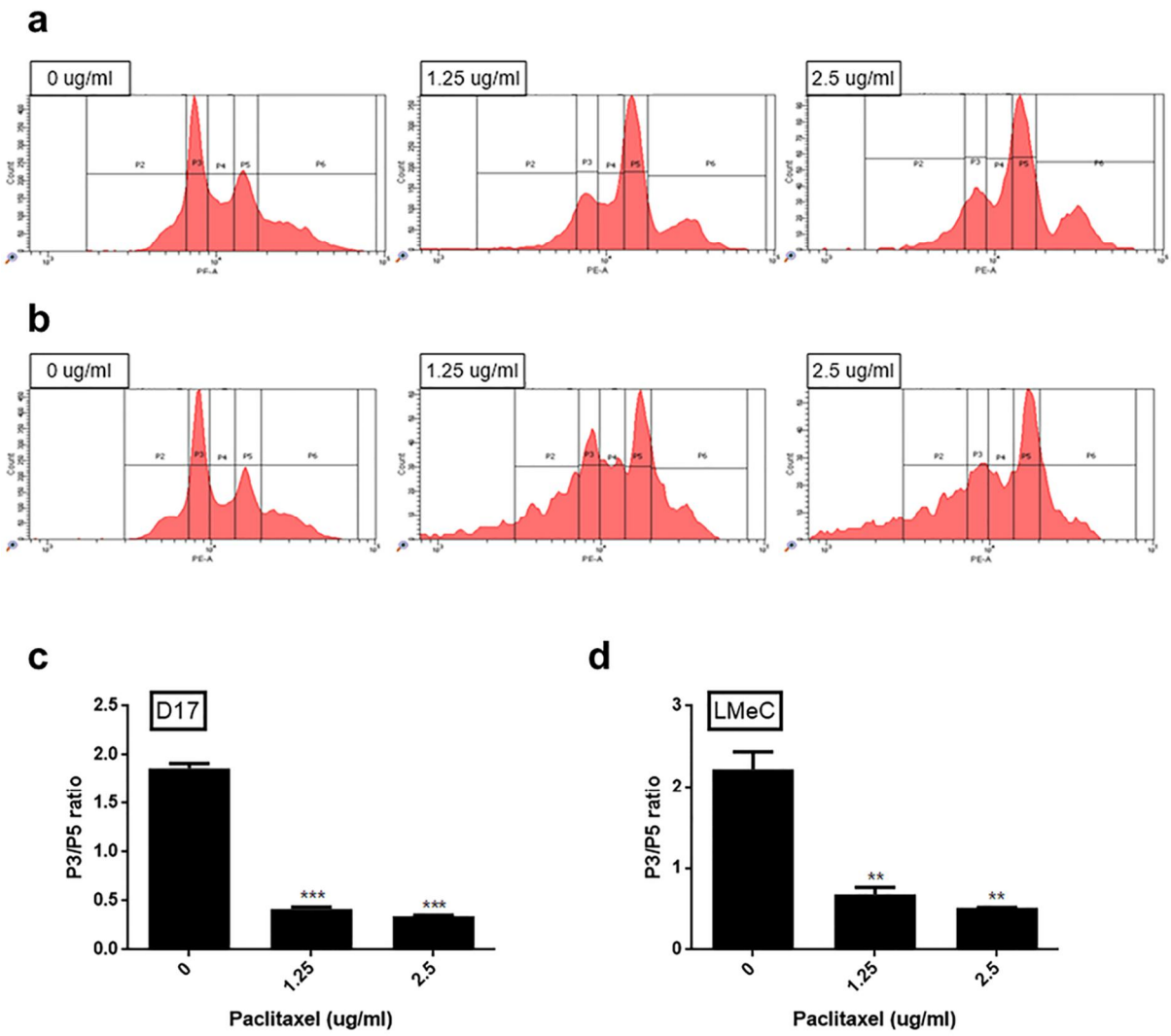

Figure 2. The cell cycle analysis of D17 and LMeC cells treated with paclitaxel after incubation for 24 h. (a) The cell cycle analysis of D17 cells treated with different paclitaxel concentrations (0, 1.25, and 2.5 μg/mL). (b) The cell cycle of LMeC treated with different paclitaxel concentrations (0, 1.25, and 2.5 μg/mL). (c) P3/P5 ratio of D17 cells. (d) The P3/P5 ratio of LMeC cells. All experiments were independently conducted in triplicate. The data are presented as the mean ± standard deviation. ** $P < 0.01$, *** $P < 0.001$.

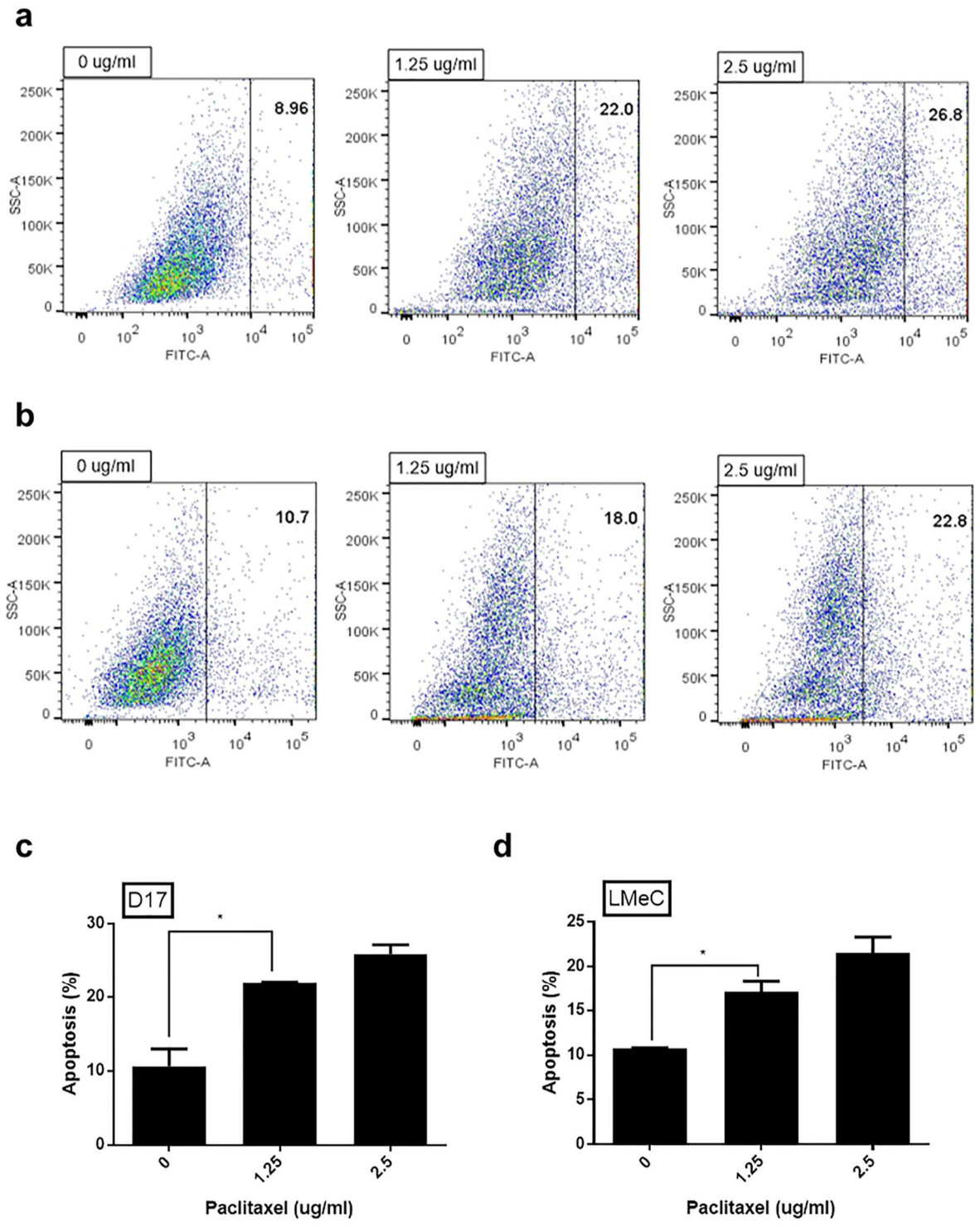

Figure 3. D17 and LMeC cells were treated with paclitaxel for 24 h and stained with annexin V. (a) The induction of apoptosis in D17 cells treated with 0, 1.25, 2.5 μg/mL

paclitaxel. (b) The induction of apoptosis in LMeC treated with 0, 1.25, 2.5 μg/mL paclitaxel. (c) The rate of apoptosis rate in D17 cells after 24 h of paclitaxel treatment. (d) The rate of apoptosis in LMeC cells after 24 h of paclitaxel treatment. All experiments were independently conducted in triplicate. The data are presented as the mean ± standard deviation. * $P < 0.05$, *** $P < 0.001$.

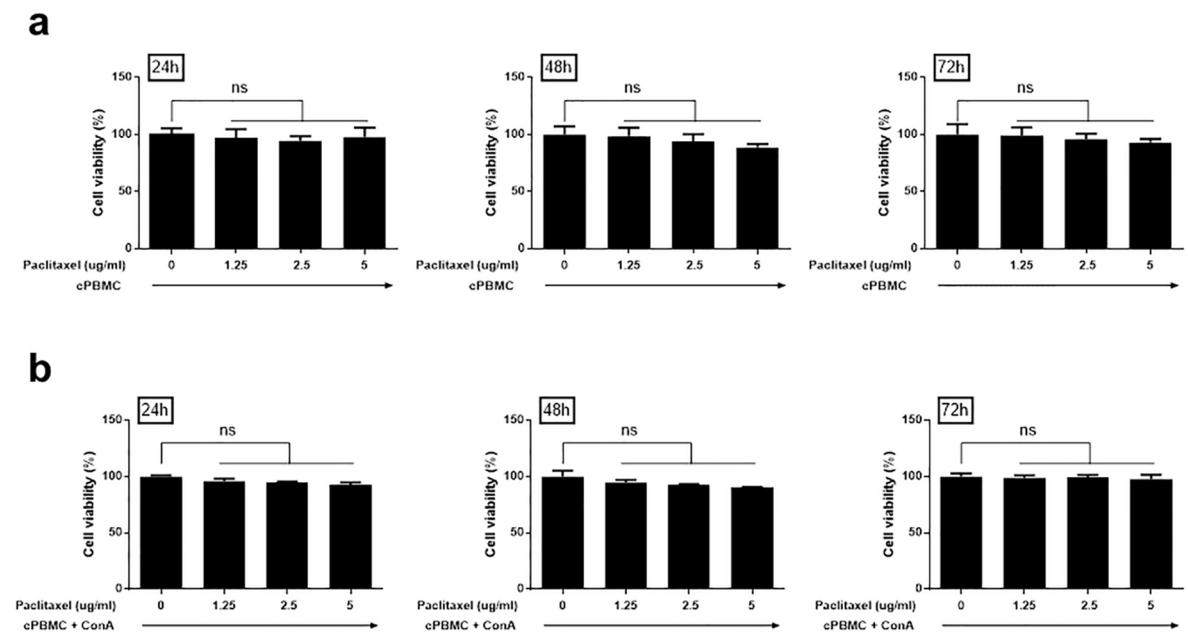

Figure 4. MTT assay for PBMCs treated with various concentrations of paclitaxel. (a) The viability of PBMCs treated with paclitaxel. (b) The viability of PBMCs treated with paclitaxel after the addition of ConA. All experiments were independently repeated in triplicate. The data are presented as the mean ± standard deviation.

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국문 초록

개의 골육종 및 흑색종 세포주에서의 파클리탁셀의 항암 효과

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개에서 유선 종양, 비만세포종, 편평상피암종, 골육종, 흑색종을 포함하는 고형 종양은 생명을 위협하는 질환 중 하나이다. 파클리탁셀은 사람과 개의 고형 종양에서 사용되는 탁센(taxane) 계열의 항암 물질로써 최근 새로운 형태의 경구용 파클리탁셀이 개발되었으나 아직까지 개의 악성 종양에 대한 항암 효과는 알려져 있지 않다. 이번 연구의 목적은 개의 골육종 및 흑색종 세포주에서의 파클리탁셀의 항암 효과를 알아보고 정상 개에서의 경구용 파클리탁셀의 임상학적 안정성을 알아보고자 하였다. 개의 골육종 및 흑색종 세포주에 대한 파클리탁셀의 항암 효과는 세포 생존율 측정 기법, 세포 주기 분석, 세포 자멸사 비율 측정 기법을 통해 확인하였다. 정상 개에서의 경구용 파클리탁셀의 안정성을 확인하기 위해 3마

리의 건강한 개에게 경구용 파클리탁셀의 치료 용량(5 mg/kg)을 8주 동안 1주일 간격으로 경구로 투약하였고, 전혈구 검사와 혈청 화학 검사를 실시하였다. 개의 골육종(D17) 및 흑색종(LMeC) 세포주에 파클리탁셀을 처리한 실험 결과 세포 증식율이 유의적으로 감소하였다. 또한 유세포분석을 통해 파클리탁셀이 개의 골육종 및 흑색종 세포주의 세포 주기를 정지시키고, 세포 자멸사를 유도한다는 것을 확인하였다. 경구용 파클리탁셀을 투약한 건강한 개에서는 부작용으로 간주할 만한 임상 증상이 관찰되지 않았고, 혈액 검사 결과는 모두 정상 범위 내로 확인되었다. 본 연구 결과는 경구용 파클리탁셀이 개의 골육종 및 흑색종을 포함한 개의 악성 종양에서 새로운 항암 물질로 적용될 수 있을 것으로 기대된다.

주요어: 경구용 파클리탁셀, 개 골육종, 개 흑색종

학번: 2015-23158