EUR. J. ONCOL.; Vol. 19, n. 4, pp. 240-248, 2014

Enhancement of tumor radio-response by vinorelbine *in vitro* and *vivo* in mice lung tumor xenografts

Chunling Gao, Xiaofeng Cheng, Xiaoliang Wang, Beibei Zhang, Jiao Zhang
ChengGong Hospital, Xiamen University/174th Hospital of PLA, Xiamen, Fujian, China

Summary. *Objective:* The aim of the experiment was to explore the optimal radioenhancement effect of vinorelbine on human lung adenocarcinoma. *Methods:* After combination treatment of 0.1 or 1nM vinorelbine with or without irradiation, cell viability was determined using clonogenic assays. Genotoxic potential of vinorelbine (NVB) and radiation (RT) alone, as well as in interaction, was determined in 973 cells using the alkaline comet assay. A male nude mouse lung tumor xenograft model was established by injecting 973 cells into nude mice. The nude mice were randomly divided into six groups: control group, 10Gy group, 20Gy group, vinorelbine group, vinorelbine + 10Gy group, vinorelbine + 20Gy group (n=8 mice per group). Vinorelbine was injected at 2 mg/kg. Tumors were measured every other day using vernier calipers. The tumor volume and growth delay were calculated. Enhancement factor (EF) was used to evaluate the radiation enhancement. *Results:* Vinorelbine had a significant effect on clonogenic survival in response to radiation in 973 cell lines at the higher dose of 1nM. The combination of vinorelbine and radiation caused a significant increase in tail moment compared to vinorelbine and radiation alone. Tumor growth inhibition in the vinorelbine + RT group was higher than in the radiation group or vinorelbine group ($p < 0.05$). The values of EF were 1.1 and 1.2, respectively. The longest time of tumor growth delay was 28 days in the vinorelbine + 20Gy group. *Conclusions:* Vinorelbine possesses radioenhancing properties for lung adenocarcinoma.

Key words: lung adenocarcinoma, vinorelbine, radiotherapy, radiation enhancement

«Potenziamento della radioterapia su tumore con vinorelbina testata in xenotrapianti di tumore polmonare tramite studi in vivo e in vitro su topi»

Riassunto. *Scopo*: Lo scopo dello studio è di esplorare l'effetto ottimale dato dal potenziamento della radioterapia con vinorelbina su adenocarcinoma polmonare umano. *Metodi*: dopo trattamento combinato di vinorelbina alla concentrazione di 0.1 o 1 nM con o senza irradiazione, è stata determinata la vitalità cellulare mediante saggi clonogenici. Il potenziale genotossico della vinorelbina (NVB) e della sola radiazione (RT), come anche la loro interazione, è stata determinata in 973 cellule utilizzando il saggio alcalino comet. Tramite l'iniezione di 973 cellule in topo maschio di tipo nudo è stato creato un modello di xenotrapianto tumorale polmonare. I topi di tipo nudo sono stati divisi in 6 gruppi: controllo, 10Gy, 20Gy, Vinorelbina, vinorelbina + 10Gy, vinorelbina + 20Gy (N= 8 topi per gruppo). La vinorelbina è stata iniettata alla concentrazione di 2 mg/Kg. I tumori insorti sono stati misurati a giorni alterni utilizzando dei calibri. Sono stati calcolati il volume della massa e il ritardo della crescita del tumore. Il fattore di potenziamento (EF) è stato utilizzato per valutare il miglioramento dato dalle radiazioni. *Risultati:* la vinorelbina ha avuto un effetto significativo sulla

This work was supported by grants from the Scientific and Technological Innovation Project of Nanjing Military Region (No. 08MA069)

sopravvivenza clonogenica in risposta a radiazioni in 973 linee cellulari quando somministrata all'alta dose di 1nM. La combinazione di vinorelbina e radiazioni ha portato ad un significativo aumento del parametro "tail moment" se paragonato alla sola somministrazione di vinorelbina o radiazioni. L'inibizione della crescita del tumore nel gruppo con vinorelbina +radiazioni era più alta rispetto al gruppo con solo radiazioni o vinorelbina ($p<0.05$). I valori EF erano rispettivamente di 1.1 e 1.2. Il più lungo intervallo di tempo nel rallentamento della crescita tumorale è stato di 28 giorni nel gruppo con vinorelbina + 20Gy. *Conclusioni:* la vinorelbina possiede la capacità di migliorare l'effetto della radioterapia nell'adenocarcinoma polmonare.

Parole chiave: adenocarcinoma polmonare, vinorelbina, radioterapia, potenziamento delle radiazioni

Introduction

A series of new chemotherapeutic agents with potential for multimodality therapy have become available for the treatment of non-small cell lung cancer (NSCLC) over the last 10 years (1). These drugs include gemcitabine and pemetrexed, antitubulin vinorelbine, and topoisomerase inhibitors such as irinotecan, analogs of platinum.

Results from these phase III studies support the use of concurrent platinum-based chemotherapy and radiotherapy (RT) in preference to RT alone or sequential chemotherapy-then-RT (2). Concurrent chemoradiotherapy with cisplatin and vinorelbine was a commonly used regimen for locally advanced NSCLC (3, 4). Naito *et al.* (3) demonstrated encouraging efficacy and safety results for this therapy in inoperable stage III NSCLC. The combination of cisplatin and vinorelbine was the most active of all the schedules adopted (5). Compared with cisplatin and vindesine, cisplatin and vinorelbine can improve the tumor control rate and prolong NSCLC patients' survival time (6).

There have been reports of a model of human A549 lung adenocarcinoma xenograft in SCID mice, where a combination of cryotherapy (nitrous oxide) with chemotherapy (vinorelbine) enhanced cell death by necrosis and apoptosis (7, 8).

Edelstein *et al.* study showed that vinorelbine can potentiate the antitumor effects of radiation and that the potentiation is cell cycle-dependent by fractional survival (9). Compared to radiation followed by drug exposure, cells treated with vinorelbine before RT obtained a better radiosensitising effect.

Currently, radiation therapy is one of the most common definitive treatment options for localized lung cancer. Although this mode of therapy is often effective, its success is far from assured. Radiation resistance causes local recurrence of the tumors. It is obvious that improving RT would have a significant positive impact on the overall success of therapy. The development of radiosensitizing agents could improve survival and quality of life, thus benefiting patients.

Radiosensitizers include traditional chemotherapeutic agents, which are widely used clinically and are considered to improve the local-regional effects of radiotherapy (10), as well as hypoxia-targeting drugs, molecular targeting agents, tumor vasculature targeting agents, etc. Typical radiosensitizers include nitromidazole, 5-gfluorouracil, analogs of platinum, gemcitabine, vinorelbine and DNA topoisomerase I-targeting drugs, avastin, endostatin, O_2, isosorbbide dinitrate, etc.

The objective of our experiment was to explore the optimal radioenhancing effect of vinorelbine on human lung adenocarcinoma. Observations were made on the radiosensitizing effects of vinorelbine *in vitro* on 973 cells (NSCLC) by fractional survival. *In vivo*, the enhancement factor was calculated according to nude mouse lung tumor xenograft growth delay. The radioenhancement by vinorelbine was to be evaluated against the enhancement factor.

Materials and methods

Animals and tumors

Male nude mice were used for this study. The care and use of the animals were in accordance with the guidelines and regulations of Xiamen University. 973 cells were grown in 1640 culture plates containing me-

dium supplemented with 10% fetal calf serum (FCS). Cells were routinely subcultured twice a week and maintained in a humidified incubator with 5% CO_2 at 37°C. 973 xenografts were generated by inoculating 973 cells subcutaneously into the right thigh of male nude mice. For radiation a linear accelerator (Varian Co., USA) was used.

Vinorelbine was diluted in 0.9% NaCl solution. *In vivo*, experiments were performed using vinorelbine at the dose of 2 mg/kg, which was prepared in 0.9% NaCl solution.

Clonogenic cell survival

The radiosensitivity effect of vinorelbine on 973 cells was assessed using standard clonogenic survival assays. Three days before irradiation, cells were plated in 25-cm^2 flasks in growth medium. In the RT+vinorelbine group cells were irradiated (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy), and then vinorelbine was added to growth medium at 0.1 or 1nM. In the vinorelbine + RT group, vinorelbine was added to cell growth medium at 0.1 or 1 nM, and then cells were irradiated (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy). Colonies were allowed to develop for 10-14 days. Cell survival curves were fitted using a linear-quadratic equation.

Cell cycle detection

Cells were plated in 25cm^2 flasks in growth medium. In the RT+vinorelbine group, cells were irradiated (6 Gy) and then vinorelbine was added to growth medium at 0.1 or 1nM. In the vinorelbine + RT group, vinorelbine was added to cell growth medium at 0.1 or 1nM, and then cells were irradiated (6 Gy). At 0, 4, 16, and 24 h after radiation, medium was collected to recover floating cells, attached cells were harvested by trypsinization and mixed with the pool of floating cells. Cells were stained with Propidium Iodide (PI) and analyzed on a flow cytometer (Coulter, America). At least 10,000 cells were counted. The proportion of cells at different phases was gated, the percentage of cells in the G2 phase was calculated using the software Multicycle (Pheonix, America).

Alkaline Comet assay

In the RT+vinorelbine group, cells were incubated with vinorelbine for 24 h and irradiated. 0.5, 1, 2, 4 and 24 hours after irradiation, cells were harvested, resuspended and mixed with 1% low-melting-point agarose at 37°C. The mixture was placed on glass slides by a straw. The glass slides were then placed in an ice-box for 4 min. After hardening, the glass slides were immersed in pre-chilled lysis buffer [2.5 mol/L NaCl, 100 mmol/L EDTA (pH 10), 10 mmol/L Tris, 1% Triton X-100 and 10% DMSO] at 4°C for 1 h. The glass slides were washed in distilled water, placed side by side on a horizontal gel chamber, and submerged in freshly made alkali buffer [300 mmol/LNaOH/1 mmol/L EDTA (pH13)] for 40 minutes followed by electrophoresis at 25 V (0.86 V/cm), 300 mA for 20 minutes at 4°C to detect both single stranded DNA breaks. The glass slides were rinsed in distilled water for 20 minutes at 4°C. After staining with PI (0.5 g/L for 20 min at 4°C), comets were assessed by fluorescence microscope (×10 objective, Olympus) and analyzed using the Comet Score software.

In vivo xenograft growth delay

Male nude mouse lung tumor xenograft models were established by injecting 973 cells into nude mice. Male nude mice bearing 973 xenografts were randomly divided into six groups: the control group, 10 Gy group, 20 Gy group, and vinorelbine group, vinorelbine+10 Gy group, and vinorelbine+20 Gy group (n=8 mice per group). Vinorelbine was injected at a concentration of 2 mg/kg. Nude mice in the control group received 0.9% NaCl solution only. Nude mice were injected intraperitoneally with vinorelbine or 0.9% NaCl solution. 24 hours after treatment with vinorelbine, nude mice in the 10 or 20 Gy groups and the vinorelbine + RT group were irradiated.

The nude mice were put into custom-built insulated chambers on the linear accelerator for radiation (8 in each box). Xenografts were irradiated using 6 MV-X ray from the linear accelerator. Mice were shielded by a lead block except for the tumor-bearing right hindlimb. In the boxes, the mice were restrained, but not anesthetized during radiation.

Three orthogonal xenograft diameters were measured with vernier calipers every other day until 40 days after the nude mouse lung tumor xenograft models were established. Nude mice were closely observed for any occurrence of toxicity right until the end.

The effect of the treatment on tumor growth delay (absolute growth delay: AGD) was defined as the time in days for tumors to reach 0.2 cm^3 in the treated group minus the mean time to reach 0.2 cm^3 in the untreated control group. The Normalized Tumor Growth Delay (NGD) was defined as the time in days for tumors to reach 0.2 cm^3 in mice treated by the drug + RT minus the time in days for tumors to reach 0.2 cm^3 in the group treated by the drug alone. The enhancement factor of the tumor radioresponse was obtained by dividing NGD by the AGD caused by radiation (11).

Statistical analysis

Results were expressed as means + SE for comparison of means, a Mann-Whitney U-Test Calculator was used (a=0.05). All statistical analyses were performed using SPSS11.5. $P<0.05$ was considered to indicate a statistically significant result.

Results

Influence of radiation and vinorelbine on clonogenic survival

The influence of vinorelbine on clonogenic survival after radiation was studied at two drug concentrations, 0.1 and 1nM. Whereas 0.1nM vinorelbine had no significant radiosensitising effect on clonogenic survival in 973 cell lines, there was radiosensitising effect on clonogenic survival by 1nM vinorelbine before or after RT (Fig. 1, Table 1). When 973 cells were treated with 1nM vinorelbine before RT, a clearer radiosensitising effect was obtained. Survival fraction at 2 Gy (SF2) is 0.865 for 973 cells. The SER of 1nM vinorelbine +RT was 1.295(>1). The SER of RT+1nM vinorelbine was 1.042(>1). The SER of 0.1nM vinorelbine +RT was 0.957(<1). The SER of RT+0.1nM vinorelbine was 0.989(<1).

Cell cycle analysis

Vinorelbine caused mitotic arrest in 973 cells. Cells were radiated at 6 Gy with or without vinorelbine. Vinorelbine (1nM) was added 24 hour before

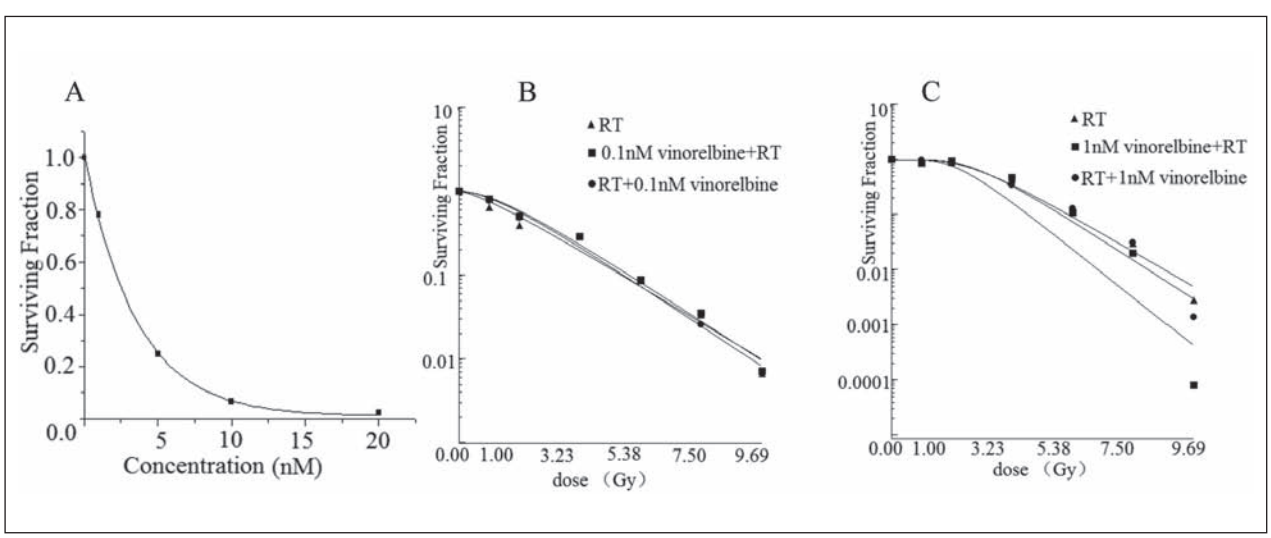

Figure 1. Clonogenic survival analysis of radiation with or without vinorelbine for 973 cells. Vinorelbine had no significant effect on clonogenic survival in response to radiation in 973 cell lines at the lower dose of 0.1 nM, but there was an increase in a component of the survival curve at the higher dose of 1nM. A) Surviving Fraction of cells treated with gradient concentrations of vinorelbine (range, 0 to 20 nM) for 24 h. B) Surviving Fraction of cells treated with gradient RT, 0.1nMvinorelbine+RT, RT +0.1nMvinorelbine. C) Surviving Fraction of cells treated with gradient RT, 1nMvinorelbine+RT, RT+1nMvinorelbine.

Table 1. Surviving Fraction parameter values of 973 Cells after radiation with or without vinorelbine at the lower doses of 0.1 nM and 1nM.

Treatment	SER	Dq	SF2	D_0	N
RT1		0.820	0.498	1.990	1.510
0.1nM vinorelbine +RT	0.957	1.393	0.586	1.854	2.120
RT+0.1nM vinorelbine	0.989	1.308	0.563	1.810	2.060
RT2		2.772	0.865	1.360	7.670
1nM vinorelbine +RT	1.295	2.279	0.759	0.990	9.990
RT+1nM vinorelbine	1.042	2.831	0.888	1.230	9.990

SER is defined as the mean inactivation dose (RT)/mean inactivation dose (vinorelbine+RT). SF, surviving fraction; D_0, mean lethal dose; Dq,quasi-threshold dose.N, extrapolation number. N, D_0, Dq and SF2 were calculated according to the survival curves.

radiation. Samples were collected at 0, 4, 16 and 24 h after radiation. The population of cells in different phases was assessed by flow cytometric assay with propidium iodide staining and the percentage of cells in the G2 phase is shown in Fig. 2, and Table 2.

Table 2. 973 cells were blocked at G2 phase when treated with RT + vinorelbine (Mean±SD).

	0 h	4 h	16 h	24 h
Control	10.0±0.7	13.7±1.4	12.5±0.7	16.5±1.5
1nM vinorelbine	16.0±0.4	15.0±0.5	12.0±0.3	16.4±1.6
RT	36.1±0.6	20.9±0.4	25.3±1.1	21.6±0.9
1nM vinorelbine+RT	39.2±0.4	30.6±0.5	28.0±0.8	26.7±1.0

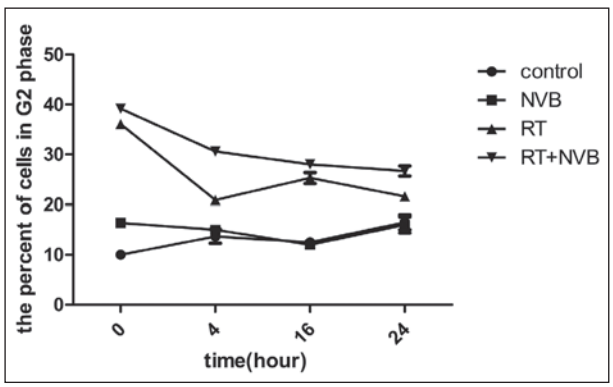

Figure 2. 973 cells blocked at G2 phase when treated with RT + vinorelbine (Mean±SEM). *P* value analysis indicated that the percent of cells in G2 phase between control and RT+NVB was statistically significant (*P*=0.021). *P* value analysis indicated that the percent of cells in G2 phase between control and RT was statistically significant (*P*=0.021). *P* value analysis indicated that the percent of cells in G2 phase between control and NVB was not differential statistically (*P*=0.564). The possible reason was the low vinorelbine concentration or the cell line. Simoens *et al.* (12) study showed that a statistically significant concentration-dependent G2/M block on CAL-27 and ECV304 was observed after 24 h vinorelbine incubation.

Alkaline Comet assay

Fig. 3 shows the results of the alkaline comet assay on 973 cells following exposure to single vinorelbine and RT as well as vinorelbine + RT. After 1 h of treatment, a significant increase in tail moment was noted upon exposure to 1nM of vinorelbine alone and RT alone, as well as vinorelbine + RT when compared to untreated cells ($P<0.05$). Vinorelbine + RT caused a significant increase in tail moment compared to vinorelbine and radiation alone ($P<0.05$). The genotoxic activity of vinorelbine alone and vinorelbine + RT on 973 cells was time-dependent.

The common condition of tumor-bearing mice

Mice in the radiation group alone and vinorelbine + RT group ate food normally and moved normally: there was no ulcer on the right thigh. All the untreated tumors grew rapidly. Two mice in the control group and two in the vinorelbine group were killed early because of abscesses affecting their ability to move. Typically, studies were terminated by the 40th day when the control xenograft tumors had exceeded 0.8 cm^3.

Xenograft tumor growth curve

The data of the xenograft tumor volume were analyzed. The xenograft tumor growth of nude mice in the control group and the vinorelbine group was nearly

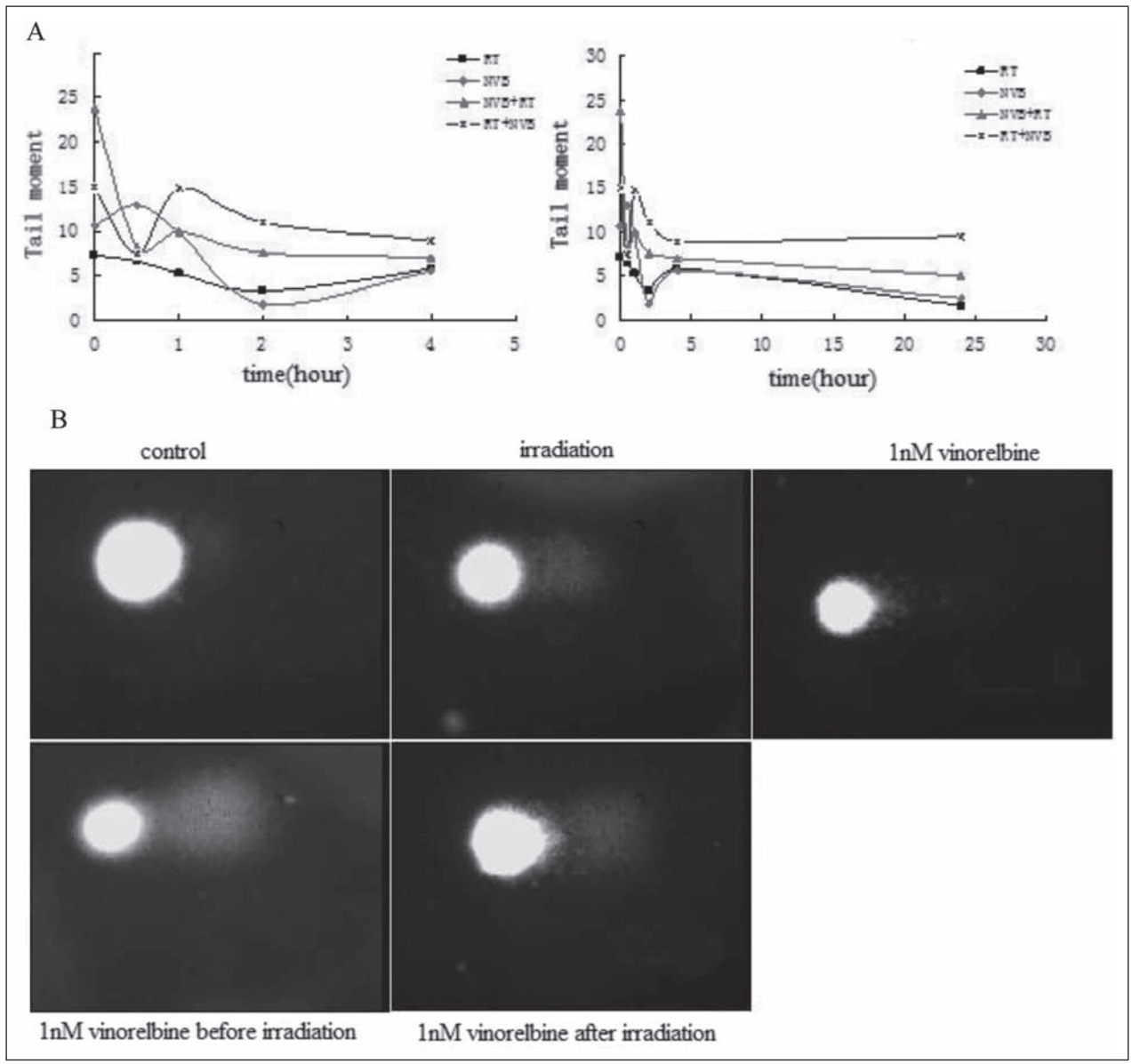

Figure 3. DNA damage represented as tail moment in 973 cells after 4 24 h of combined treatment with vinorelbine + RT. (A) The vinorelbile+RT curve represents a significant synergistic effect ($P<0.05$). (B) 973 cells' DNA damage was detected by Alkaline Comet Assay. The result shows that DNA damage in the 1nM vinorelbine before radiation group was heaviest of all. It represented a significant synergistic effect.

the same. Compared with them, the tumor growth of mice in the radiation group was slow. Xenograft tumor growth of mice in the 20 Gy group was slower than mice in the 10 Gy group. Compared with mice treated with 10 Gy or 20 Gy, xenograft tumor growth in mice from the vinorelbine+RT group was markedly slow (Fig. 4).

Tumor growth delay and enhancement factor

The radiosensitising effect of vinorelbine was evaluated on lung adenocarcinoma tumors in mice. For evaluation of antitumor effectiveness, tumor growth delay was used. The enhancement factors among mice in the vinorelbine +10 Gy group and the vinorelbine +

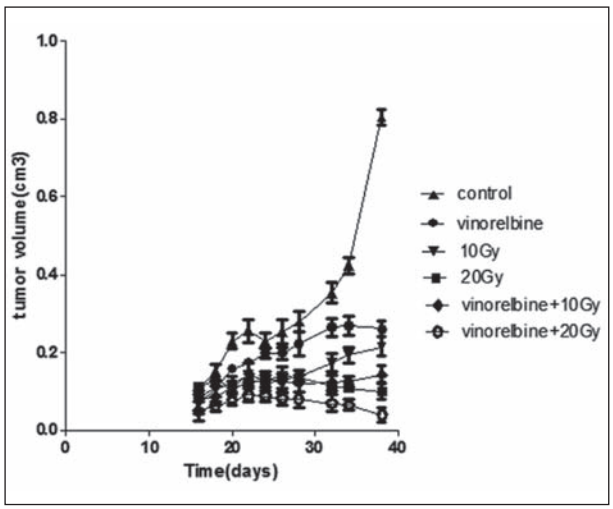

Figure 4. Tumor growth delay assay on 973 xenografts treated with radiation, vinorelbine and vinorelbine + 20 Gy, vinorelbine + 10 Gy.

Table 3. Effect of vinorelbine on radioresponse of 973 tumors, influence of time interval between vinorelbine administration and radiation delivery.

Treatment	Time in days that tumors take to grow from 0.1 to 0.2 cm^3	AGD	NGD	EF
Control	18.0±1.4			
Radiation				
10 Gy	24.0±1.1	6		
20 Gy	38.0±1.3	20		
Vinorelbine	22.0±1.1	4		
Vinorelbine + 10 Gy	29.0±1.8	11	7	1.1
Vinorelbine + 20 Gy	46.0±1.5	28	24	1.2

AGD was defined as the time in days for tumors to reach 0.2 cm^3 in the treated group minus the mean time to reach 0.2 cm^3 in the untreated control group. NGD was defined as the time in days for tumors to reach 0.2 cm^3 in mice treated by the drug + RT minus the time in days for tumors to reach 0.2 cm^3 in the group treated by the drug alone. EF was obtained by dividing NGD by the AGD caused by radiation

20 Gy group were 1.16 and 1.2 respectively. The tumor growth delay among mice in the vinorelbine + 20 Gy group was 28 days (Table 3).

Discussion

Vinorelbine is a semisynthetic vinca alkaloid that has been shown to be effective both as a single agent and in combination therapies for the treatment of breast and non-small-cell lung cancer (13-16). Cisplatin plus vinorelbine regimen is a good candidate for combination with concurrent radiotherapy because of its efficacy and safety. These results are highly promising, being even better than other concurrent chemotherapy studies, with very good tolerance and little toxicity (17).

Most radiosensitizers induce radiosensitization by the following pathways: (1) increased primary radiation damage [e.g., BrdU (18)], (2) inhibition of DNA damage repair [e.g., irinotecan (19)], (3) cell cycle arrest [e.g., YM155 (20)], (4) enhanced apoptosis [e.g., Paclitaxel (21)], (5) altered tumor microenvironment, decreased hypoxia [e.g., TX-402(22)], etc.

A series of reports had demostrated the radiosensitising effect of vinorelbine, but the exact mechanisms of radiosensitising by vinorelbine were unknown. Recent data have shown the possible mechanisms of radiosensitization by vinorelbine, as described below.

In a preclinical report described by Fukuoka *et al.*, SBC-3 cells (human SCLC cells) were sensitized to radiation by VRL and the possible mechanism of the VRL-induced radiosensitization may in part be associated with impairment of DNA repair following radiation-induced DNA damage. It was hypothesized that the disruption of microtubule integrity in SBC-3 cells by VRL might partly inhibit p53 transport to the nucleus, resulting in impairment of p53-mediated DNA repair following radiation-induced DNA damage (23).

Fukuoka *et al.* reported that PC9 (NSCLC cells) were sensitized to radiation by vinorelbine by causing accumulation of cells in the G2/M phase of the cell cycle: a specific increase in apoptotic cell death occurred upon entering the cell cycle after G2/M arrest (24).

Edelstein *et al.* study showed that vinorelbine can potentiate the antitumor effects of radiation and that the potentiation is cell cycle-dependent, with maximum effect when cells are in the G2 phase (9). The results showed that the mechanisms of radioenhancement by vinorelbine were cell line dependent.

There was also a report about clear evidence of vascular damage in tumors taken from mice treated with both vinorelbine (8 mg/kg^{-1}) and vinflunine (50 mg/kg^{-1}): the vascular damage was dose-dependent and happened 24h after treatment with vinorelbine (25). It could be a possible mechanism of radiosensitization.

Our study showed that 24 h incubation with vinorelbine before or after radiation resulted in a dose-dependent potentiation of radiation according to the surviving cell fraction and the clonogenic survival result. When 973 cells were treated with 1nM vinorelbine before RT, a clearer radiosensitising effect was obtained. In this study, the effect on clonogenic survival in response to radiation by vinorelbine in 973 cell lines was dose-dependent, and the timing was also critical.

According to the tumor growth curve, the enhancement factors of the vinorelbine+10 Gy group and the vinorelbine+20 Gy group were 1.16 and 1.2, respectively. The tumor growth delay in the vinorelbine+20 Gy group was 28 days. Our investigations revealed that vinorelbine (1nM) possessed radioenhancing properties for 973 cells. The dose-dependent response of mouse lung tumor xenografts to radiation exposure was also shown. When mice were treated by vinorelbine +20 Gy, the tumor growth stopped. 20 Gy proved a high dose to nude mouse lung tumor xenografts when they were radiated at one and the same time. Thus concurrent chemoradiotherapy was supposed to be effective in patients with locally advanced NSCLC (26). Furthermore, we noticed that mice bearing tumors were healthy when they were treated with vinorelbine + RT.

There are various ways to detect DNA damage, including neutral or alkaline comet assay, measurement of γ H2AX foci (markers of DNA double strand breaks) by immunoflurescence or western blot, pulsed field gel electrophoresis etc. The alkaline single cell gel electrophoresis (comet) assay is widely used due to its sensitivity, speed, flexibility, reliability and low cost (27, 28). We hence used the alkaline comet assay to detect any single strand breaks (SSB).

We noticed that 973 cells exhibited a significantly different radioresponse to RT in SSB repair when they were treated with RT+ vinorelbine compared to just vinorelbine. Vinorelbine + RT caused a significant increase in tail moment compared to vinorelbine and radiation alone. We believe that the slower repair kinetics of 973 cells was the major contributing factor toward radiosensitization of vinorelbine.

Simoens *et al.* (12) reported that a statistically significant concentration-dependent G2/M block was observed after 24 h incubation with vinorelbine compared to controls. The effect was concentration-dependent. Our research showed that the percentage of cells in the G2 phase between controls and the NVB group was not differential in terms of statistics. The possible reason was the low vinorelbine concentration or the different cell line.

In a future study we would like to focus on the change of protein expression level in 973 cells after vinorelbine+RT treatment which might explain why vinorelbine achieved radiation enhancement in 973 cells.

In conclusion, vinorelbine enhanced the effect of radiation both *in vitro* and *in vivo*. Timing and dose appeared to be two critical factors in producing the combination effects. The potential mechanism of radiosensitization might be connected with slower SSB repair. These preclinical data suggest that vinorelbine could be administered with RT to improve clinical efficacy, especially in human lung adenocarcinoma.

References

1. Kawahara M, Kris MG, Green M, *et al.* New drug and multi-modality combinations in the treatment of advanced non-small cell lung cancer. Semin Oncol 2001; 28: 1-4.
2. Cosaert J, Quoix E. Platinum drugs in the treatment of non-small-cell lung cancer. Br J Cancer 2002; 87, 825-33.
3. Naito Y, Kubota K, Nihei K, *et al.* Concurrent chemoradiotherapy with cisplatin and vinorelbine for stage III non-small cell lung cancer. J Thorac Oncol 2008; 3: 617-22.
4. Sekine I, Nokihara H, Sumi M, *et al.* Docetaxel consolidation therapy following cisplatin, vinorelbine, and concurrent thoracic radiotherapy in patients with unresectable stage III non-small cell lung cancer. J Thorac Oncol 2006; 1: 810-5.
5. Bria E, Cuppone F, Cecere F, *et al.* Adjuvant chemotherapy for non-small cell lung cancer. J Thorac Oncol 2007; 2: S7-S11.
6. Le Chevalier T, Brisgand D, Douillard JY, *et al.* Randomized study of vinorelbine and cisplatin versus vindesine and cisplatin versus vinorelbine alone in advanced non-small cell lung cancer: results of a European multicenter trial including 612 patients. J Clin Oncol 1994; 12: 360-7.
7. Forest V, Peoc'h M, Ardiet C, *et al.* In vivo cryochemotherapy of a human lung cancer model. Cryobiology 2005; 51: 92-101.
8. Forest V, Peoc'h M, Campos L, *et al.* Benefit of a combined treatment of cryotherapy and chemotherapy on tumour growth and late cryo-induced angiogenesis in a non-small-cell lung cancer model. Lung Cancer 2006; 54: 79-86.

9. Edelstein MP, Wolfe LA, Duch DS. Potentiation of radiation therapy by vinorelbine (Navelbine) in non-small-cell lung cancer. Semin Oncol 1996; 23: 41-7.
10. Cooper JS, Ang KK. Concomitant chemotherapy and radiation therapy certainly improves local control. Int J Radiat Oncol Biol Phys 2005; 61: 7-9.
11. Milas L, Fujii T, Hunter N, *et al.* Enhancement of tumor radioresponse in vivo by gemcitabine. Cancer Res 1999; 59 (1): 107-14.
12. Simoens C, Lardon F, Pauwels B, *et al.* Comparative study of the radiosensitizing and cell cycle effects of vinflunine and vinorelbine, in vitro. BMC Cancer 2008; 8: 65.
13. Gridelli C. The ELVIS trial: A phase III study of single-agent vinorelbine as first-line treatment in elderly patients with advanced non-small cell lung cancer. Elderly Lung Cancer Vinorelbine Italian Study. Oncologist 2001; 6: 4-7.
14. Gridelli C, Gallo C, Shepherd FA, *et al.* Gemcitabine plus vinorelbine compared with cisplatin plus vinorelbine or cisplatin plus gemcitabine for advanced non-small-cell lung cancer: A phase III trial of the Italian GEMVIN Investigators and the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2003; 21: 3025-34.
15. Zelek L, Barthier S, Riofrio M, *et al.* Weekly vinorelbine is an effective palliative regimen after failure with anthracyclines and taxanes in metastatic breast carcinoma. Cancer 2001; 92: 2267-72.
16. Le Chevalier T, Brisgand D, Soria JC, *et al.* Long term analysis of survival in the European randomized trial comparing vinorelbine/cisplatin to vindesine/cisplatin and vinorelbine alone in advanced non-small cell lung cancer. Oncologist 2001; 6: 8-11.
17. Provencio M, Isla D, Sanchez A, *et al.* Inoperable stage III non-small cell lung cancer: Current treatment and role of vinorelbine. J Thorac Dis 2011; 3: 197-204.
18. Dextraze ME, Cecchini S, Bergeron F, *et al.* Reaching for the other side: generating sequence-dependent interstrand cross-links with 5-bromodeoxyuridine and gamma-rays. Biochemistry 2009; 48: 2005-11.
19. Kunimoto T, Nitta K, Tanaka T, *et al.* Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothec in, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 1987; 47: 5944-7.
20. Qin Q, Cheng H, Lu J, *et al.* Small-molecule survivin inhibitor YM155 enhances radiosensitization in esophageal squamous cell carcinoma by the abrogation of G2 checkpoint and suppression of homologous recombination repair. Journal of Hematology & Oncology 2014; 7: 62.
21. Zhang AL, Russell PJ, Knittel T, *et al.* Paclitaxel enhanced radiation sensitization for the suppression of human prostate cancer tumor growth via a p53 independent pathway. Prostate 2007; 67: 1630-40.
22. Nagasawa H, Uto Y, Kirk KL, *et al.* Design of Hypoxia-Targeting Drugs as New Cancer Chemotherapeutics. Biol Pharm Bull 2006; 29 (12): 2335-42.
23. Fukuoka K, Arioka H, Iwamoto Y, *et al.* Mechanism of vinorelbine-induced radiosensitization of human small cell lung cancer cells. Cancer Chemother Pharmacol 2002; 49: 385-90.
24. Fukuoka K, Arioka H, Iwamoto Y, *et al.* Mechanism of the radiosensitization induced by vinorelbine in human non-small cell lung cancer cells. Lung Cancer 2001; 34: 451-60.
25. Holwell SE, Hill BT and Bibby MC. Anti-vascular effects of vinflunine in the MAC 15A transplantable adenocarcinoma model. Br J Cancer 2001; 84 (2): 290-5.
26. Ishida K, Hirose T, Yokouchi J, *et al.* Phase II study of concurrent chemoradiotherapy with carboplatin and vinorelbine for locally advanced non-small-cell lung cancer. Molecular and Clinical Oncology 2014; 2: 405-10.
27. Tice RR, Agurell E, Anderson D, *et al.* Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000; 35: 206-21.
28. Fairbairn DW, Olive PL, O'Neill KL. The comet assay: a comprehensive review. Mutat Res 1995; 339: 37-59.

Received: 10.9.2014
Accepted: 7.10.2014
Address: Dr. Chunling Gao
ChengGong Hospital, Xiamen University/174th Hospital of PLA, Xiamen, Fujian, 361003, China
E-mail: chunlinggaocn@yahoo.com