Role of gambogic acid and NaI^{131} in A549/DDP cells

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Abstract. Resistance to platinum in tumor tissue is a considerable barrier against effective lung cancer treatment. Radionuclide therapy is the primary adjuvant treatment, however, the toxic side effects limit its dosage in the clinical setting. Therefore, the present study aimed to determine whether an NaI^{131} radiosensitizer could help reduce the toxic side effects of radionuclide therapy. *In vitro* experiments were conducted to determine whether NaI^{131} can inhibit platinum resistance in A549/DDP cells, which are cisplatin-resistant non-small cell lung cancer cells, and whether gambogic acid (GA) is an effective NaI^{131} radiosensitizer. Cell proliferation following drug intervention was analyzed using MTT and isobolographic analysis. Apoptosis was assessed by flow cytometry. In addition, the mechanisms of drug intervention were analyzed by measuring the expression of P-glycoprotein (P-gP), B cell lymphoma 2 (Bcl-2), Bcl2-associated X protein (Bax) and P53 using western blot analysis and immunocytochemistry. According to isobolographic analysis, a low concentration of NaI^{131} combined with GA had a synergistic effect on the inhibition of A549/DDP cell proliferation, which was consistent with an increased rate of apoptosis. Furthermore, the overexpression of Bax, and the downregulation of P-gP, P53 and Bcl-2 observed demonstrated the potential mechanism(s) of NaI^{131} and GA intervention. NaI^{131} may induce apoptosis in A549/DDP cells by regulating apoptosis-related proteins. A low concentration combination of NaI^{131} and GA was able to significantly inhibit A549/DDP cell proliferation and induce cell apoptosis. Thus, the two drugs appear to have a synergistic effect on apoptosis of A549/DDP cells.

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Introduction

Lung cancer is the leading cause of cancer-associated mortality in the USA. In 2015, there was an estimated 221,200 novel cases of lung and bronchial cancer diagnosed, and 158,040 mortalities were attributed to lung cancer (1). In China, lung cancer-associated mortality accounts for >20% of all cancer mortalities (2). Currently, chemotherapy regimens containing platinum are the primary therapies for advanced non-small cell lung cancer (NSCLC) (3). However, the efficacy of chemotherapy is influenced by the resistance to platinum in the tumor tissue, leading to variations in efficacy between individuals (4). Several mechanisms have been proposed to explain the resistance of cancer cells to chemotherapy (5-7). It has been demonstrated in previous studies that resistance to platinum in lung cancer cells could be enhanced by P-glycoprotein (P-gP) expression, which is upregulated by the multi-drug resistance-1 gene (8,9). In addition, other studies have demonstrated that an imbalance in the expression of cell apoptosis-related regulatory proteins, such as P53 and Bcl2-associated X protein (Bax), can inhibit tumor cell apoptosis, causing lung cancer cells to become resistant to platinum (10,11).

Radionuclide therapy is an important method of tumor irradiation. The combination of ^{125}I implantation in tumor tissue and chemotherapy has an important role in the clinical treatment of lung cancer, and this regimen has a significantly positive efficacy compared with chemotherapy alone (12). However, to the best of our knowledge, no studies have been performed regarding the therapeutic mechanism of radionuclides in drug-resistant tumor cells. Highly concentrated radioactivity also results in toxic side effects for patients, such as myelosuppression and aplastic anemia (13), limiting the dosage of radionuclide therapy that can be used in the clinic. Therefore, it is critical to identify an effective radiosensitizer to reduce the side effects of radionuclide therapy.

Gambogic acid (GA) is extracted from gamboge and the monomers are purified. The molecular formula of GA is $C_{38}H_{44}O_9$ (molecular weight, 628.34 g/mol) and its chemical structure is known. GA is an effective anti-tumor agent that has been shown to have multiple effects on several types of solid human tumors *in vitro* and *in vivo* (14,15). For example, studies have demonstrated that GA can upregulate the expression of Bax and P53, and downregulate the expression of B cell

lymphoma-2 (Bcl-2), inhibiting tumor cell apoptosis (16,17). GA has also been implicated in several mechanisms of cisplatin resistance (18). Furthermore, it has exhibited detectable effects on patients with lung cancer, colorectal cancer and renal cell carcinoma (19-21). Thus, GA has been approved for evaluation in a phase II clinical trial for NSCLC in China (approval no. 2004L00333) (22).

In vitro experiments were conducted in the current study to determine whether NaI^{131} is able to inhibit platinum resistance in cisplatin-resistant A549/DDP NSCLC cells and whether GA is an effective NaI^{131} radiosensitizer during the treatment of A549/DDP cells. The present study also aimed to determine the potential mechanism(s) of GA-associated NaI^{131} radiosensitization in lung cancer.

Materials and methods

Materials. Human cisplatin-resistant NSCLC cells were provided by Dr Zhibo Hou (Department of Pneumology, Nanjing Chest Hospital, Medical School of Southeast University, Nanjing, China). MTT and mouse monoclonal anti-P-gP (catalog no. ab3366), anti-Bcl-2 (catalog no. ab692), anti-Bax (catalog no. ab77566), anti-P53 (catalog no. ab28), anti-β-actin (catalog no. ab8226) antibodies, and goat anti-mouse IgG secondary antibody (catalog no. ab6789) were acquired from Abcam (Cambridge, MA, USA). The secondary antibody used for immunocytochemistry, goat anti-mouse IgG/horseradish peroxidase-conjugated antibody, was purchased from ZSGB-BIO (Beijing, China).

Cell culture. Cells were cultured in RPMI-l640 medium supplemented 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Cells were digested with 0.25% EDTA-phosphate-buffered saline (PBS) during the logarithmic proliferation phase. Cell suspensions were transferred to frozen vessels and stored at 4˚C for 30 min, -20˚C for 1 h, and -80˚C overnight in liquid nitrogen.

MTT assay. A549/DDP cells in the logarithmic proliferation phase were randomly assigned to the NaI^{131} intervention group, GA intervention group or control group. The A549/DDP cells were seeded into 96-well plates ($1x10^4$-$1x10^5$ cells/well). The NaI^{131} group was treated with NaI^{131} 5.7, 11.4, 17.1, 22.8, 28.5 or 34.1 MBq; the GA group was treated with GA 0.5, 1.0, 1.5, 2.0 or 3.0 μg/ml; and the control group was treated with equal volumes of PBS. The cells were incubated in a standard cell culture incubator at 37˚C with 5% CO_2. After 48 h of cell culture, 5 mg/ml MTT (20 μl/well) was added to the media and the cells were additionally incubated for 4 h. Dimethylsulfoxide (150 μl; Sigma-Aldrich, St. Louis, MO, USA) was added to the cells in each of the wells after the media was removed, and the cells were further incubated for 10 min. The optical density (OD) of each well was measured using a microplate reader (Multiskan™ GO Microplate Spectrophotometer; Thermo Fisher Scientific, Inc.) at 560 nm. All experiments were performed in triplicate, and results were analyzed according to the following formula: Cell inhibitory rate (%) = (1- OD test group / OD control group) x 100.

Treatment with 5.7 MBq NaI^{131} and 0.2 μg/ml GA was also performed; these drug concentrations were empirically determined based on the various doses of NaI^{131} and GA evaluated, which were then used to measure the half maximal inhibitory concentrations (IC_{50}) of the two drugs. Graphical representations of the isobolographic analysis, performed as previously described (23,24), were used to determine whether NaI^{131} and GA synergistically inhibit the proliferation of A549/DDP cells.

Apoptosis and protein detection

Cell treatment. A549/DDP cells in the logarithmic proliferation phase were assigned to the NaI^{131}, GA, NaI^{131} combined with GA or control group. The A549/DDP cells were seeded into 96-well plates ($1x10^4$-$1x10^5$ cells/well). Based on IC_{50} values, the NaI^{131} group was treated with 17.5 MBq NaI^{131}; the GA group was treated with 1.5 μg/ml GA; and the combined treatment group was treated with 17.5 MBq NaI^{131} and 0.2 μg/ml GA. The control group was treated with an equal volume of PBS.

Apoptosis analysis by flow cytometry. After 48 h of culture (at 37˚C with 5% CO_2), cell apoptosis was analyzed using Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) iodide kit (Kaiji Bio, Co., Nanjing, China). Adherent and floating cells were harvested and disaggregated to a single-cell suspension. Staining was performed according to the manufacturer's protocols. The data were analyzed by flow cytometry using CXP2.2 software (Beckman Coulter, Inc., Brea, CA, USA).

Bcl-2, P-gP, Bax and P53 immunocytochemistry. After 48 h of culture (at 37˚C with 5% CO_2), $5x10^5$ cells/ml were placed on Culture Slides (BD Biosciences, Bedford, MA, USA). The slides were washed with PBS, and fixed using 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, incubated with 3% hydrogen peroxide and then maintained in a blocking solution composed of PBS with 3% albumin from bovine serum (Sigma-Aldrich). The slides were subsequently incubated overnight at 4˚C with specific primary antibodies against Bcl-2, P-gP, Bax and P53 (dilution, 1:200). Subsequently, the cells were washed with PBS, and incubated with diluted biotinylated secondary antibody and then with VECTASTAIN® ABC Reagent (Dako Italia, Milan, Italy). Finally, the slides were washed, incubated with peroxidase substrate solution until desired stain intensity developed (Peroxidase/DAB; Dako Italia), rinsed in tap water, counterstained with Mayer's hematoxylin and mounted with BioMount. The results of immunocytochemical staining was negative (-), weak positive (+) and positive (++) and strong positive (+++). Analysis of the OD data was performed using ImageJ software (version 1.37; National Institutes of Health, Bethesda, MD, USA). The results were expressed as the mean ± standard deviation (%).

Western blot analysis. Cell protein was extracted using 500 μl radioimmunoprecipitation assay lysis buffer with 5 μl phenylmethylsulfonyl fluoride and protease inhibitor (Abcam, Cambridge, UK). Total proteins were quantified using the Bicinchoninic Acid Assay (Beyotime Institute of Biotechnology, Shanghai, China), according to the manufacturer's protocols. Total protein (20 μg) was loaded onto an 8% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). The membrane was incubated for 1 h at 25˚C in 5% skimmed dried milk and then washed three times for 5 min at 25˚C using blocking buffer. Subsequently, the membrane was incubated overnight at 4˚C with specific primary antibodies for P-gp, p53, Bax and Bcl-2. All antibodies

were diluted to 1:1,000. Subsequent to being washed three times with TBST for 5 min each, the membranes were incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (dilution, 1:5,000). In order to evaluate protein expression accurately, β-actin was used as an internal standard. Band intensity was analyzed with ImageJ software, and protein expression was presented as the ratio of the protein band intensity to β-actin in the same blot.

Statistical analysis. Every experiment was performed in triplicate, and each group was comprised of three duplicate wells. Statistics were analyzed with SPSS (version 19.0; IBM SPSS, Armonk, NY, USA). All data are presented as the mean ± standard deviation. Graphs were plotted using Microsoft Office Excel 2007 (Microsoft Inc., Redmond, WA, USA). Differences between groups were analyzed by one-way analysis of variance or independent sample *t*-tests. The SNK test are used at the second stage of the analysis of variance if the null hypothesis was rejected. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cell proliferation inhibition rates per group

Inhibitory effect of NaI^{131} or GA alone on A549/DDP cells. The inhibition of A549/DDP cell proliferation after 48 h of treatment with NaI^{131} is shown in Table I. The half maximal inhibitory concentration (IC_{50}) value was 17.54±1.86 MBq (Fig. 1).

The cell proliferation inhibition ratio of A549/DDP cells after 48 h of GA treatment is shown in Table II. The IC_{50} value was 1.46±0.07 μg/ml (Fig. 2).

Inhibitory effect of NaI^{131} combined with GA on A549/DDP cells. NaI^{131} and GA administered singly would kill a limited number of cells. Preincubation of A549/DDP cells with the usual GA dose (1.46 μg/ml) followed by their exposure to NaI^{131} (17.54 MBq) results in the rapid death of all cells. Therefore, in principle, a combination consisting of a low dose of GA and appropriately adjusted NaI^{131} dosage would be effective. Consequently, doses of 5.7 MBq NaI^{131} and 0.2 μg/ml GA were empirically determined for the combined treatment. The effects of various doses of NaI^{131} and GA on cell proliferation were determined by the MTT assay, and the proliferation inhibition rates of the two groups were calculated. The proliferation inhibition rates of A549/DDP cells after treatment with NaI^{131} (5.7 MBq) and GA (various doses) for 48 h are shown in Table III. The IC_{50} value of GA was 0.22±0.03 μg/ml. The cell proliferation inhibition rates of A549/DDP cells after 48 h of treatment with GA (0.2 μg/ml) and NaI^{131} (various doses) are indicated in Table IV. The IC_{50} value of NaI^{131} was 7.14±0.88 MBq.

Based on the aforementioned data, two composite points were plotted: i) 5.7 MBq NaI^{131} and 0.22±0.03 μg/ml GA; and ii) 0.2 μg/ml GA and 7.14±0.88 MBq NaI^{131}. In A549/DDP cells, the IC_{50} of NaI^{131} was 17.54±1.86 MBq (point A) and the IC_{50} of GA was 1.46±0.07 μg/ml (point B) (Fig. 3). According to the isobolographic analysis, the straight line connecting A and B is the locus of points (dose pairs) that will produce this effect in a simply additive combination. Two composite points are in the

Table I. Cell proliferation inhibition ratio of A549/DDP cells following treatment with various doses of NaI^{131}.

NaI^{131}, MBq	Inhibition ratio of A549/DDP cells, %[a]
5.7	18.12±11.16
11.4	29.31±7.61
17.1	50.43±9.00
22.8	64.52±2.32
28.5	65.82±5.67
34.1	70.78±2.29

[a]Data are presented as the mean ± standard deviation.

Table II. Cell proliferation inhibition ratio of A549/DDP cells following treatment with various doses of GA.

GA, μg/ml	Inhibition ratio of A549/DDP cells, %[a]
0.5	7.67±1.53
1.0	29.00±2.65
1.5	53.33±2.08
2.0	68.33±2.08
3.0	78.00±1.00

[a]Data are presented as the mean ± standard deviation. GA, gambogic acid.

Table III. Cell proliferation inhibition rate of A549/DDP cells following treatment with NaI^{131} (5.7 MBq) and GA (various doses).

GA, μg/ml	Inhibition ratio of A549/DDP cells, %[a]
0.1	33.10±4.30
0.2	40.30±6.00
0.3	52.83±8.56
0.4	75.16±3.47

[a]Data are presented as the mean ± standard deviation. GA, gambogic acid.

Table IV. Cell proliferation inhibition rate of A549/DDP cells following treatment with GA (0.2 μg/ml) and NaI^{131} (various doses).

NaI^{131}, MBq	Inhibition ratio of A549/DDP cells, %[a]
5.7	44.82±5.52
11.4	59.89±3.07
17.1	73.72±3.69

[a]Data are presented as the mean ± standard deviation. GA, gambogic acid.

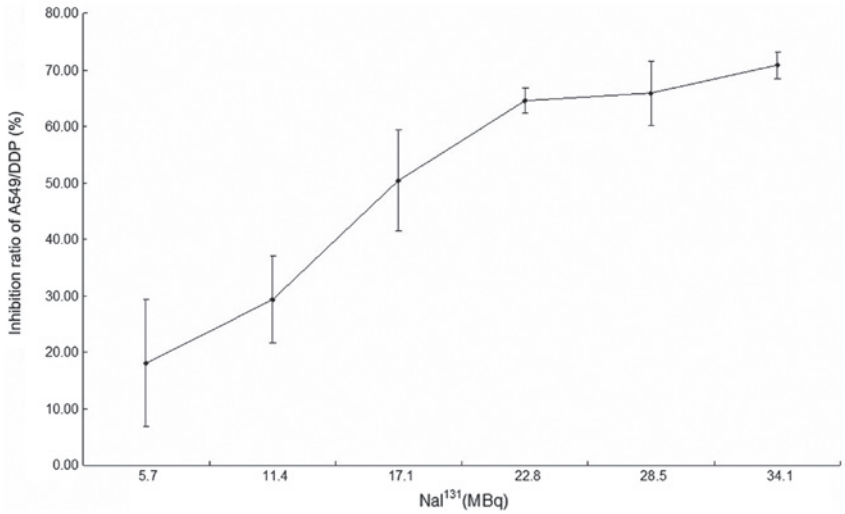

Figure 1. A549/DDP cells were treated with 5.7, 11.4, 7.1, 22.8, 28.5 or 34.1 MBq of NaI^{131} for the indicated times. Loss of cell viability was assessed by MTT assay. Data are the means of six independent experiments; bars, standard deviation.

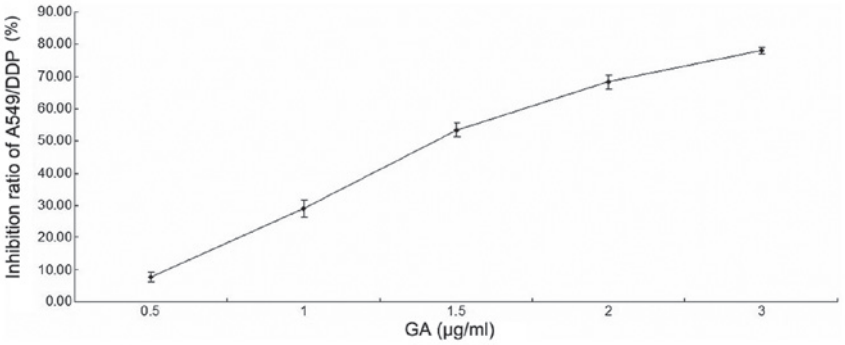

Figure 2. A549/DDP cells were treated with 0.5, 1.0, 1.5 ,2.0 or 3.0 μg/ml of GA for the indicated times. Loss of cell viability was assessed by MTT assay. Data are the means of six independent experiments; bars, standard deviation. GA, gambogic acid.

lower left from the hypotenuse, suggesting that NaI131 and GA synergistically inhibit A549/DDP cell proliferation.

Apoptosis detection analysis. Annexin V-FITC and PI flow cytometry revealed that the cell count ratios in the Q4 quadrant were significantly increased in the NaI^{131,} GA and combined treatment groups compared with the control group, indicating an increase in the rate of early apoptosis. Furthermore, compared with the control group, the cell count ratio in the Q2 quadrant (late apoptosis) was significantly increased in the NaI^{131}, GA and combined treatment groups. In addition, the number of cells in early or late apoptosis in the combined treatment group were increased significantly compared with those in the NaI^{131} and GA alone groups. Total apoptosis rates also showed the same patterns (Table V; Fig. 4).

Intracellular P-gP, Bcl-2, Bax and P53 protein expression detected by immunocytochemistry. The P-gP protein was detected by immunostaining with granular coloring observed in the cell membrane and cytoplasm surrounding the nucleus. Expression was strongly positive (+++) in the control cells, positive (+) in the NaI^{131} and GA cells, and moderate (±) in the combined treatment cells. P53 protein was primarily detected in the nucleus. Its expression was positive (++) in the control group, weakly positive (+) in the NaI^{131} and GA groups, and moderate (±) in the combined treatment group. Bax protein

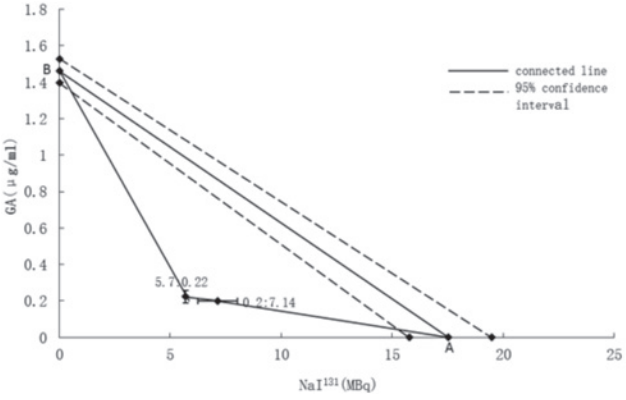

Figure 3. Isobolographic analysis of GA and NaI^{131}. The three points on the x axis are the IC_{50} values of NaI^{131} with 95% CI. The three points on the y axis are the IC_{50} values of GA with 95% CI. The dotted lines indicate the 95% CI, and the area between the dotted lines is the confidence limit. The solid line connects the two IC_{50} values. The curved solid line was determined experimentally, derived from the IC_{50} of combinations of NaI^{131} and GA. GA, gambogic acid; CI, confidence interval.

Table V. Cell apoptosis was analyzed using flow cytometry following 48 h of cell culture.

Treatment group	Apoptosis, %		
	Early	Late	Total
Control	1.74±0.60	2.21±1.33	3.95±1.24
NaI^{131}	10.39±1.17[a]	4.39±0.96[a]	14.78±1.91[a]
GA	13.38±1.96[a]	5.73±1.58[a]	19.11±1.03[a]
Combined	20.06±3.02[b]	11.49±2.34[b]	31.55±5.32[b]

Data are presented as the mean ± standard deviation. [a]P<0.05 vs. control group; [b]P<0.01 vs. control NaI^{131} and GA groups. GA, gambogic acid.

Table VI. Expression of P-gP, Bcl-2, P53 and Bax protein expression as detected by western blot assay.

Treatment group	Protein expression level			
	P-gP	Bax	Bcl-2	P53
Control	0.83±0.08	0.15±0.01	0.89±0.20	0.67±0.11
NaI^{131}	0.55±0.05[a]	0.32±0.02[a]	0.59±0.13[a]	0.54±0.09
GA	0.74±0.07[a]	0.29±0.02[a]	0.66±0.15[a]	0.57±0.09[a]
Combined	0.34±0.03[b]	0.85±0.06[b]	0.28±0.06[b]	0.36±0.06[b]

Data are presented as mean ± standard deviation. [a]P<0.05 vs. control group; [b]P<0.01 vs. control NaI^{131} and GA groups. GA, gambogic acid; P-gP, P-glycoprotein; Bax, Bcl2-associated X protein; Bcl-2, B cell lymphoma-2.

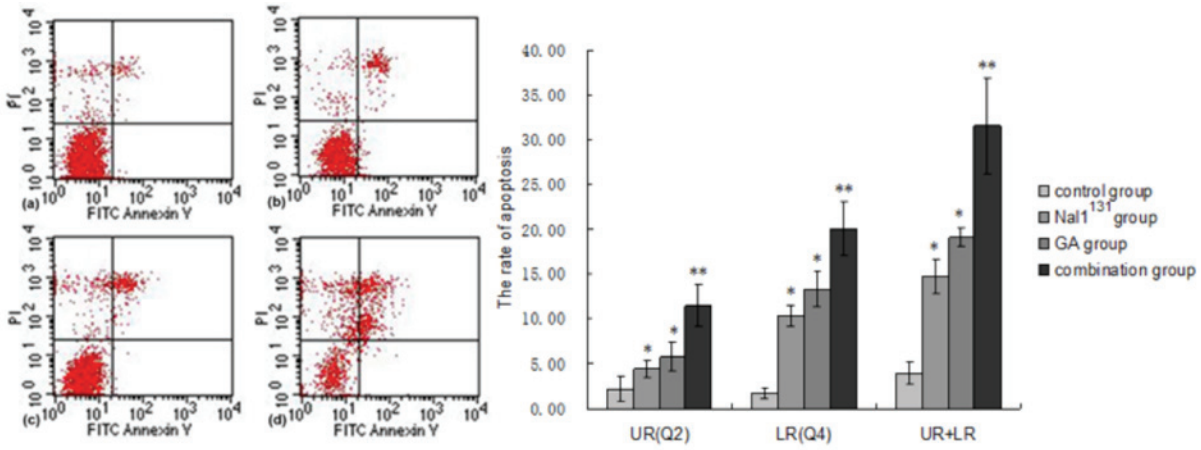

Figure 4. Cell apoptosis was analyzed using flow cytometry after 48 h of cell culture for the control, NaI^{131}, GA and combined treatment group. Data are presented as representative graphs in the left panel. Q2 and Q4 flow cytometry data are quantified in the right panel. UL, dead cells; UR, late apoptosis; LL, cell survival; LR, early apoptosis. Data are presented as the mean ± standard deviation. *P<0.05 vs. control group; **P<0.05 vs. control, NaI^{131} and GA groups. PI, propidium iodide; FITC, fluorescein isothiocyanate; GA, gambogic acid; UL, upper left; UR, upper right; LL, lower left; LR, lower right.

was detected with cytoplasmic staining. It showed moderate expression (±) in the control group, weakly positive expression (+) in the NaI^{131} and GA groups, and positive expression (++) in the combined treatment group. Bcl-2 protein was detected by immunostaining with granular coloring primarily observed in the cell membrane and cytoplasm. Its expression was positive (++) in the control group, weakly positive (+) in the NaI^{131} and GA groups, and moderate (±) in the combined treatment group (Fig. 5).

P-gP, Bcl-2, Bax and P53 protein expression detected by western blot analysis. The protein expression levels of P-gP, Bcl-2 and P53 significantly decreased, while the expression level of Bax protein significantly increased in the combined treatment group compared with the control, NaI^{131} and GA groups. Similarly, P-gP, Bcl-2 and P53 levels significantly decreased and Bax levels significantly increased compared in the NaI^{131} and GA alone groups compared with the control group (Table VI; Fig. 6).

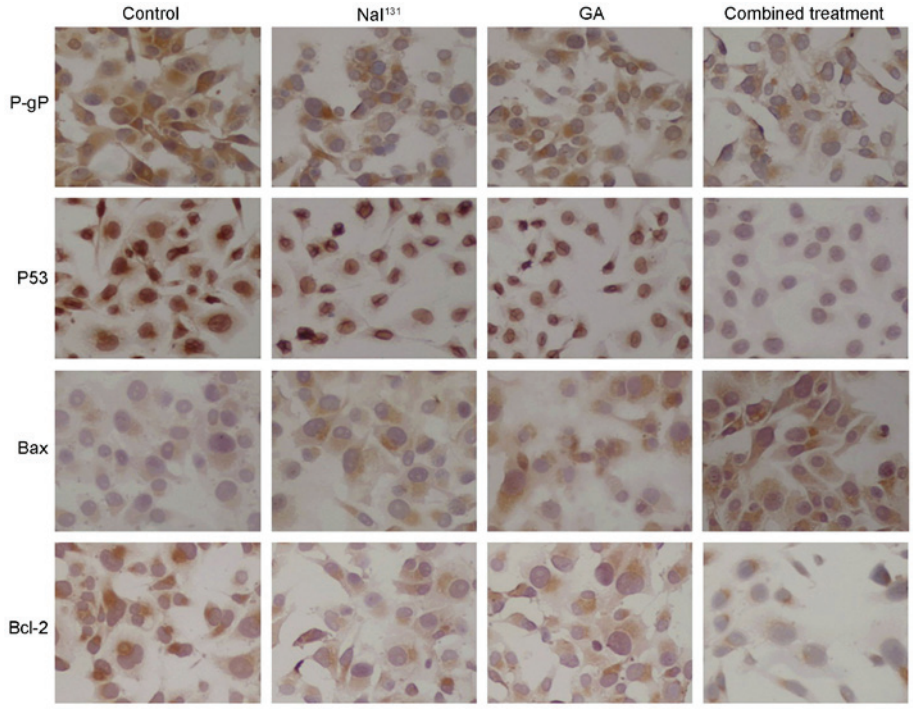

Figure 5. Protein expression of Bcl-2, P-gP, Bax and P53 detected using immunocytochemistry in the control, NaI^{131}, GA and combined treatment group. GA, gambogic acid; P-gP, P-glycoprotein; Bax, Bcl2-associated X protein; Bcl-2, B cell lymphoma-2.

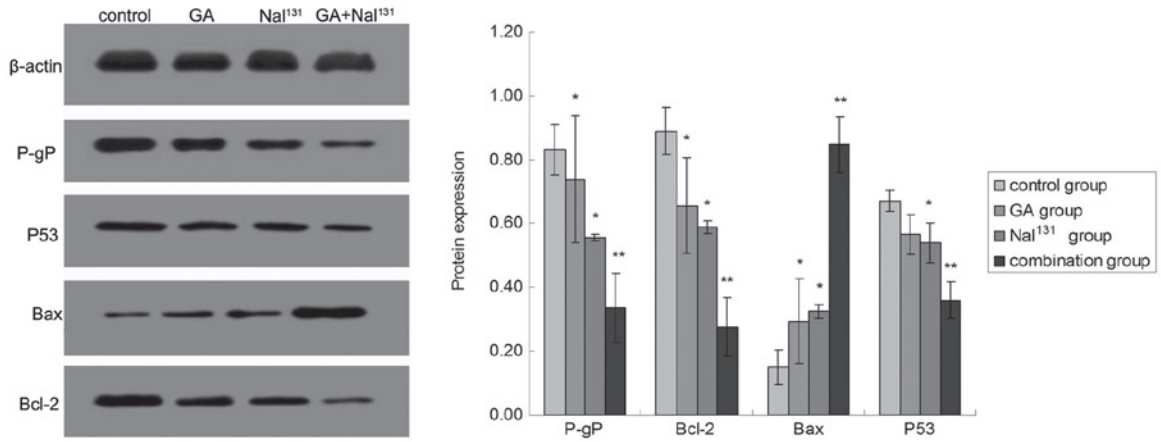

Figure 6. P-gP, Bcl-2, Bax and P53 protein expression detected by western blot analysis. The blot is presented in the left panel and quantification is shown in the right panel. *P<0.05, **P<0.05 vs. control group, NaI^{131} group and GA group. Data are presented as the mean ± standard deviation. GA, gambogic acid; P-gP, P-glycoprotein; Bax, Bcl2-associated X protein; Bcl-2, B cell lymphoma-2.

Discussion

Previous studies have demonstrated that cisplatin resistance in NSCLC cells occurs via several mechanisms (8-11). These mechanisms of resistance primarily involve the abnormal expression of drug transporters and drug metabolizing enzymes in cells (25), increased DNA damage repair (26), abnormal regulation of cell cycle and apoptosis-related protein expression (27).

In general, implanting radioactive particles into tumor tissue is supplemented with chemotherapy (28,29). Radiation can induce the apoptosis of tumor cells through activation of the P53 and Bax genes, and inhibition of the Bcl-2 gene (30-33). The current results suggest that NaI^{131} also induces apoptosis by regulating P53, Bax and Bcl-2 expression in A549/DDP cells. However, due to their lack of specificity and strong binding force, radionuclides can cause extensive damage to non-targeted cells. The presence of nuclear particles in the normal tissue surrounding the tumor lesion commonly causes localized and systemic side effects during nuclide particle therapy (13,34-36), which limits their value in the clinic. Several studies have found that GA can promote lung cancer cell apoptosis by multiple mechanisms (37-40). Furthermore, GA has been reported to act as a sensitizer for chemotherapy. A previous analysis of the ability of GA to sensitize lung cancer for chemotherapy demonstrated positive results (41). According to the results of the isobolographic analysis performed in the present study, we hypothesize that a combination of GA and I^{131} may have a synergistic effect on

the inhibition of A549/DDP cells. Thus, only a low dose of GA combined with I^{131} is required to significantly increase the rate of apoptosis in A549/DDP cells.

P-gP is a membrane transport protein that can efflux intracellular anticancer agents out of tumor cells, increasing a patient's resistance to antitumor drugs (42). The current results suggest that GA and I^{131} alone may decrease the expression of P-gP. In addition, compared with GA or I^{131} treatment alone, I^{131} combined with low doses of GA was better able to inhibit the expression of P-gP. Application of this treatment strategy may, therefore, further weaken the capacity of tumor cells to efflux intracellular drugs, partially reversing the multidrug resistance of cells.

It is widely accepted that the protein ratio of Bax and Bcl-2 has an important role in apoptosis. Apoptosis is considered to be promoted in cells with high Bax protein expression and inhibited in cells with high Bcl-2 protein expression. Several previous studies have also confirmed the role of both proteins in lung cancer cells (43-45). The current results suggest that in A549/DDP cells, I^{131} and GA alone are able to regulate the protein expression levels of Bcl-2 and Bax. Compared with the groups treated with each agent alone, the protein expression of Bcl-2 was significantly decreased and that of Bax was significantly increased in the I^{131} combined with GA group. Thus, we hypothesize that a low dose of GA can enhance the ability of I^{131} to regulate Bcl-2 and Bax expression, which may promote apoptosis in A549/DDP cells.

Other previous studies of lung cancer have found that wild-type P53 can promote tumor cell apoptosis (46,47), increasing the chemosensitivity of tumor cells (48,49). However, mutant P53 can promote chemotherapeutic tolerance in tumor cells via several factors, such as decreased pro-apoptotic capacity, increased DNA repair function (50,51) and upregulated P-gP expression (9,52). It has been confirmed that the wild-type P53 protein cannot be detected by immunohistochemical techniques due to its instability, rapid hydrolysis and short half-life, as well as for other unknown reasons. However, mutant P53 protein can be detected by immunohistochemical methods reasonably well due to its relative stability and longer half-life (50,53). The results of the current immunocytochemical analysis and western blot assay suggest that A549/DDP cells exhibit high P53 protein expression levels, and treatment with GA or I^{131} alone was able to reduce this expression. In addition, compared with the groups treated with each agent alone, P53 protein expression was further decreased in the I^{131} combined with GA group, suggesting that the combination of these two compounds may promote apoptosis in A549/DDP cells by regulating the expression of mutant P53.

In conclusion, the current results suggest that the effect of NaI^{131} on A549/DDP cells may promote apoptosis by increasing P53 and Bax protein expression, while reducing Bcl-2 protein expression. Treatment with NaI^{131} combined with a low dose of GA was more capable of promoting apoptosis compared with the two monotherapies. Additional protein detection suggested that the protein expression levels of P-gP, P53 and Bcl-2 were markedly decreased and the protein expression of Bax was significantly increased in the combined treatment group compared with each monotherapy group. These results suggest that low-dose GA may enhance the pro-apoptotic role of NaI^{131} administered to A549/DDP cells in cisplatin-resistant NSCLC. Therefore, low-dose GA may be a sensitizer to NaI^{131} radiotherapy, since reducing the dosage of NaI^{131} used in the clinic would reduce the toxic side effects caused by radiotherapy.

Acknowledgements

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