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*Original Research*

# Effects of ambroxol hydrochloride on concentrations of paclitaxel and carboplatin in lung cancer patients at different administration times

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**Abstract:** Our previous preliminary study revealed a synergistic effect of ambroxol hydrochloride with chemotherapeutic agents such as paclitaxel and carboplatin in lung cancer. However, the optimal conditions such as administration time and drug concentration of ambroxol hydrochloride to achieve the maximum synergistic effect remained unclear. Therefore, concentration changes of the chemotherapy drugs paclitaxel and carboplatin in the sputum were observed after ambroxol hydrochloride administration at different times in order to determine the most effective time frame of ambroxol hydrochloride administration. In this study, 470 cases of non-small cell lung cancer (NSCLC) were divided into different groups with ambroxol hydrochloride administered at different time points prior to chemotherapy, while another 171 cases received no ambroxol hydrochloride prior to chemotherapy. The results showed the concentrations of paclitaxel and carboplatin in sputum of patients treated with ambroxol hydrochloride were significantly higher than those of the control group, suggesting that ambroxol hydrochloride significantly increased the local concentrations of chemotherapeutic agents in lung tissues of NSCLC. Furthermore, the intravenous administration of ambroxol hydrochloride more than 48 hours before chemotherapy showed an optimized schedule and much greater efficacy in increasing drug concentrations than that of the control group. No statistical differences were found in the rates of grade 2 or above myelosuppression between the ambroxol intervention and control groups. Taken together, these results demonstrate that ambroxol hydrochloride administered intravenously more than 48 hours prior to chemotherapy optimally increased the concentrations of paclitaxel and carboplatin in lung tissue without significantly increasing hematologic toxicity.

***Key words:*** Non-small cell lung cancer, ambroxol hydrochloride, paclitaxel, carboplatin.

## Introduction

Lung cancer patients usually receive 2 to 4 cycles of chemotherapy following surgery, and the majority of patients benefit from adequate chemotherapy. Currently, the classical combination treatment of paclitaxel and carboplatin is used as the standard first-line chemotherapy for NSCLC. The clinical efficacy of chemotherapy drugs not only depends on the sensitivity oration in targetf tumor cells to the drugs, but also closely correlates to the drug concentration in target organs and tissues. Therefore, it is important to achieve a high target concentration with a relative low dosage of chemotherapy drugs. Ambroxol hydrochloride, a commonly used expectorant, has been clinically used in lung cancer treatment as auxiliary and routine medication. Ambroxol hydrochloride is usually administered through intravenous infusion for perioperative and/or postoperative chemotherapy. Ambroxol hydrochloride has been reported to synergize with antibiotics to enhance the concentration of antibiotics in the sputum of patients with pulmonary infections (1-3). Previously preliminary research revealed that high-dose ambroxol hydrochloride can result in better clinical effects than normal doses in perioperative lung protection in patients with lung cancer (4). Ambroxol hydrochloride is usually given to lung cancer patients at different time schedules, and little is known about the effective timing of ambroxol hydrochloride administration with chemotherapy drugs to achieve optimal effects. Thus, this study aims to investigate the concentration changes of paclitaxel and carboplatin in sputum after time-dependent administration of ambroxol hydrochloride to cancer patients, in order to empirically identify the appropriate timing of drug administration for ambroxol hydrochloride.

## Materials and Methods

### Clinical data

A total of 641 NSCLC patients, who underwent chemotherapy post surgery from May 2013 to April 2015, were selected. Of these patients, 470 cases were divided into 4 groups (A-D) based on different ambroxol hydrochloride treatment schedules prior to chemotherapy treatment; the other 171 patients were treated with chemotherapy alone and formed a control group without ambroxol hydrochloride treatment. Group A (drug administration within 12 hours before chemotherapy) contained 176 patients with 119 males and 57 females, age 49-72, median age (67.1±9.7); group B (drug administration 12-24 hours before chemotherapy) contained 84 patients, 59 males, 25 females, age 39-81, median age 64.9±15.6; group C (drug administration 24-48 hours before chemotherapy) contained 119 patients with

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83 males, 36 females, age 46-79, median age 67.4±13.4; and group D (drug administration more than 48 hours before chemotherapy) contained 91 patients with 60 males, 31 females, age 44-78, median age 66.4±11.2. The control group contained 101 males and 70 females, age 42-76, median age 65.1±11.2. Ambroxol hydrochloride (90 mg) was administered via intravenous infusion multiple times per day.

**Reagents**

Paclitaxel standard (>99%) was purchased from Shanghai Dragon Biological Drug Development Co., LTD (catalog number: LX-P-902-0904006); Docetaxel, an internal standard, was purchased from Sigma-Aldrich Co.(catalog number: Y0001452; St. Louis, MO, USA); Carboplatin standard was purchased from Sigma-Aldrich (catalog number: 41575-94-4; St. Louis, MO, USA); methanol, acetonitrile, formic acid, and chromatographic grade reagents were purchased from Fisher (Fair Lawn, NJ, USA). Medicine treatment in patients: Ambroxol hydrochloride Injection (catalog number: 12311474) was purchased from Boenhringer Ingelhelm Eapana, S.A., Paclitaxel Injection (catalog number: 1303202) was purchased from Sichuan Sunnyhope Pharmaceutical Co., LTD, Carboplatin Injection (catalog number: WB2J1307013) was purchased from Shandong Qilu Pharmacy Pharmaceutical Co., LTD.

**Instruments**

Liquid chromatography/mass spectrometry system (LC/MS/MS): Triple Quadrupole LC/MS/MS, Agilent QQQ 6420 (Agilent, USA); chromatographic column: Agilent C-18, Dim 50×2.1mm, Particle size 3.5μ (Agilent, USA); low-speed refrigerated centrifuge: Centrifuge 5810R (EPENDORF, Germany); tabletop high-speed refrigerated centrifuge: Centrifuge 5415R (EPENDORF, Germany); MassHunter workstation quantitative analysis system software (version B.01.04); MassHunter workstation LC/MS data acquisition for 6400 series triple quadrupole (version B.06.00).

**Methods**

Patients from groups A, B, C, D and the control group received 2 to 4 cycles of chemotherapy; 6 hours post treatment, deep sputum and 5 ml of venous blood were collected. Before collecting the sputum, oxygen nebulization was performed for 15 min during which the patients were encouraged to take a mild cough, in order to induce the production of sputum and to help cough out the sputum. In a semi-recumbent position, patients were then asked to take deep breaths 5 to 10 times within a 5 min interval, and their upper body was positioned forward to facilitate expectoration of the deep sputum from the lung. Sputum samples were then collected and stored at -20ºC for further analysis. Concentration changes of chemotherapy drugs in sputum after ambroxol hydrochloride treatment at different administration times were then compared and analyzed. Pretreatment of control; 100 μl normal sputum sample was mixed with paclitaxel standard solution and internal control docetaxel solution with 1 min vortex, and then centrifuged at 13000 g for 10 min, then the supernatant was collected. Carboplatin standard solution (0.5 mol/L NaOH + 2% sodium diethyldithiocarbamate) was added into 200 μl of normal sputum sample, the mixture was incubated at 60ºC for 30 min after 10 s vortex, and mixed with 700 μl acetonitrile with 30 s vortex, followed by drying with a vacufuge. The sample was resuspended with 200 μl acetonitrile, vortexed for 15 s and centrifuged at 10000 g for 5 min, and the supernatant was then kept. Pretreatment of samples: 100 μl and 200 μl sputum samples for testing were processed following the procedures of pretreatment of control, and then the supernatant was collected for further analysis. Concentrations of paclitaxel and carboplatin from the sputum were quantified by liquid chromatography/mass spectrometry (LC/MS/MS) system. Drug concentration in sputum can be measured by calculating the signal area of the curve and further converted into the corresponding concentration according to the standard curve. Mass spectrometer: Selective ion monitoring was carried out by electrospray ionization (ESI) with ESI voltage of 4000V, ESI pressure of 20 psi, and temperature 350 degrees. Selective ion monitoring (SIM) was used to detect $[M+Na]^+$ molecular ion peak of paclitaxel (m/z 876.3) with secondary fragment ions at m/z 593.3 and 308.1; $[M+Na]^+$ molecular ion peak of docetaxel (m/z 830.5) with secondary fragment ions at m/z 549.3 and 304.4; molecular ion peak of carboplatin (m/z492) with secondary fragment ions at m/z 310.9 and 88. Chromatographic column: Agilent C-18, Dim 50×2.1 mm, Particle size 3.5 μ; Agilent SBC18 4.6×50 mm (Agilent, USA); chromatographic conditions: water: acetonitrile: 0.1% formic acid (Lot number: A20471, J.T. Baker)=35:65:0.1(v/v/v) was used as the mobile phase for detecting paclitaxel with a flow rate of 0.3ml/min, and column temperature 23ºC; 0.1% formic acid and acetonitrile were used as mobile phase A and B respectively for detecting carboplatin, with the following gradient elution steps: 0 min 30% B; 0.5 min 30% B; 4.5 min 90% B; 7 min 90% B.

**Statistical analysis**

Data sets of drug concentration-time were collected using "Agilent MassHunter Quantitative Analysis" software. The data are shown as mean ± standard deviation (SD) or incident count (n) (%). Statistical significance of the data was assessed by SPSS20.0 software, differences between groups were determined with ANOVA followed by Tukey's *post hoc* test, percentage differences between groups were determined with chi-square test, and $P<0.05$ was considered statistically significant.

**Results**

**Specificity and standard curve**

Quantitative analysis of drug concentrations was performed using selective ion monitoring and the separation and detection of the compounds was successful, and was not interfered with by endogenous substances in the sputum. For paclitaxel, the regression equation incorporating response values and concentrations was Y=0.8050X+0.00083, and the coefficient of correlation (r2) was 0.9959 within the range of 2.5 to 1000 ng/ml in sputum. The standard curve equation obtained for carboplatin was Y=43.2326X+35.3321, with r2 of 0.9951 within the range of 2 to 1000 ng/ml. Concentrations and

**Table 1.** Chemotherapy drug concentrations (ng/ml) in sputum following ambroxol hydrochloride intravenous.

| Agents | Control (n=171 ) | Group A (<12h) (n=176) | Group B (12-24h) (n=84) | Group C (24-48h) (n=119) | Group D (>48h) (n=91) |
|---|---|---|---|---|---|
| Paclitaxel | 12.74±11.72 | 19.67±16.1* | 27.73±19.89* | 31.96±28.26**#$ | 37.68±34.61**#$ |
| Carboplatin | 184.13±179.38 | 302.31±257.35* | 317.21±240.77* | 436.32±329.87**#$ | 462.33±347.72**#$ |

The data are shown as mean ± standard deviation (SD). *$P < 0.05$, **$P < 0.01$ versus control group, #$P < 0.05$ versus group A, $$P <$ 0.05 versus group B, analysis between different groups were determined with ANOVA followed by Tukey's *post hoc* test.

response ratios showed a positive linear relationship.

**Effects of ambroxol hydrochloride on chemotherapy drug concentrations in lung cancer patients at different administration times**

Patients received daily intravenous infusions of 90 mg ambroxol hydrochloride beginning at different times before chemotherapy; following chemotherapy, concentrations of paclitaxel and carboplatin in the sputum of patients were measured. Our data (Table 1) showed that concentrations of chemotherapy drugs in all of the ambroxol hydrochloride treated groups were significantly higher compared with the control group (P<0.05). This suggests that ambroxol hydrochloride significantly increases the local concentration of chemotherapy drugs in the lungs of NSCLC patients. The highest drug concentrations were observed in groups D and C, followed by groups B and A. There was no statistical difference in drug concentrations between group D and group C, as well as between group B and group A, while differences between other group combinations showed statistical significance ($P$<0.05). This indicates that administration of ambroxol hydrochloride at different time results in different levels of enhancement of drug concentrations in NSCLC patients who underwent chemotherapy. The optimal time for introducing ambroxol hydrochloride intravenously was found to be 48 hours before chemotherapy (Figure 1).

**Rates of myelosuppression (grade 2 or above) in patients given ambroxol hydrochloride**

No significant difference was observed in the rates

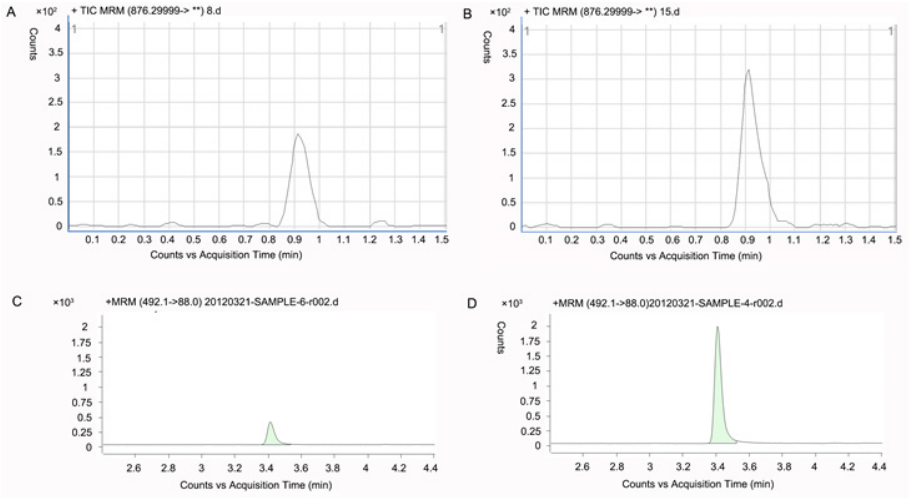


**Figure 1.** Representative LC/MS/MS spectrum of paclitaxel and carboplatin in the sputum sample. A: paclitaxel control without ambroxol hydrochloride treatment; B: paclitaxel with ambroxol hydrochloride administration for over 48h; C: carboplatin control without ambroxol hydrochloride treatment; and D: carboplatin with ambroxol hydrochloride administration for over 48h. Drug concentrations were determined by response values (counts vs acquisition time) according to the standard curve.

**Table 2.** Percentage of patients with grade 2 or above myelosuppression [n (%)].

| Group | Incident Count n (%) | | | |
|---|---|---|---|---|
| | Hemoglobin (<94g/L$^{-1}$) | Leukocyte (<3.0×10$^9$/L$^{-1}$) | Neutrophil (<1.5×10$^9$/L$^{-1}$) | Platelet (<75×10$^9$/L$^{-1}$) |
| Control (n= 171) | 27(15.3) | 59(33.5) | 65(36.9) | 28(15.9) |
| Group A (<12h) (n=176) | 25(14.2) | 54(30.7) | 61(34.7) | 31(17.6) |
| Group B (12-24h) (n=84) | 13(15.5) | 25(29.8) | 28(33.3) | 15(17.9) |
| Group C (24-48h) (n=119) | 16(13.4) | 37(31.1) | 41(34.5) | 20(16.8) |
| Group D (>48h) (n=91) | 13(14.3) | 29(31.9) | 32(35.2) | 17(18.7) |
| *P* value | 0.8971 | 0.712 | 0.821 | 0.7455 |

The data are shown as incident count n (%), percentage differences between groups were determined with chi-square test.

of grade 2 or above myelosuppression between the ambroxol hydrochloride groups and the control group (Table 2). This suggests that ambroxol hydrochloride combined with paclitaxel and carboplatin neither increased hematologic toxicity, nor caused negative effects on peripheral blood.

## Discussion

This study shows that the proper timing of ambroxol hydrochloride administration to NSCLC patients significantly enhanced the chemotherapy drug (paclitaxel and carboplatin) concentrations in sputum. The optimal administration time was shown to be 48 hours or more before chemotherapy, because the combination treatment allowed paclitaxel and carboplatin to reach desired concentrations without introducing hematologic toxicity. Our findings provide valuable insight into the clinical application of anticancer agents.

LC-MS/MS was employed in this study to quantify paclitaxel in sputum, providing fast analyzing speed (testing time of 1.5 min per sample) with high sensitivity; the minimal detection threshold was 2.5 ng/ml with high specificity (endogenous substances in the sputum did not interfere with drug determination). The pre-column derivatization by chelating sodium diethyldithiocarbamate tandem mass spectrometric determination method (based on the characteristic chemical structure of carboplatin) used in this study increased sensitivity with a detection threshold of 2 ng/ml, and the gradient elution was able to reduce interference from the sputum. The two methods described above provided accurate and reliable data for detecting the concentrations of paclitaxel and carboplatin in the lungs of cancer patients who underwent combination treatment with ambroxol hydrochloride and chemotherapy.

Ambroxol hydrochloride is an effective lung targeting expectorant, which is widely used in clinics. It pharmacologically regulates serous fluid and mucus secretion, stimulates the production of intrinsic alveolar surfactant, and increases ciliary activity to promote mucus clearance (5). Ambroxol hydrochloride also effectively enhances the anti-oxidative defense function of pulmonary tissue, decreases oxidative damage, and lowers the rates of complications after thoracotomy operations (6-8). In addition, ambroxol hydrochloride removes reactive oxygen species, reduces inflammatory cells, releases proinflammatory cytokines, subsequently alleviates alveolar epithelial tissue damage, and promotes drug absorption in lung tissue (9). Our clinical data demonstrated an almost two-fold increase of paclitaxel and carboplatin concentrations in the combination treatment group compared with the control group receiving chemotherapy alone. The above observations might be attributed to following reasons: (1) ambroxol hydrochloride modulates the secretion of serous fluid and mucus in the lung, enhancing the sputum excretion and thus improving small-airway patency and suppressing the pulmonary inflammation response; (2) ambroxol hydrochloride stimulates the activation of choline - phosphatidyl transferase, which in turn promotes pulmonary surfactant production, reduces alveolar surface tension, effectively prevents alveolar collapse, and facilitates the pulmonary transportation of drugs; and (3) ambroxol hydrochloride inhibits the release of inflammatory mediators by mast cells, overtly reduces pulmonary inflammatory responses, and enhances the enrichment of the drugs in lung tissue.

Ambroxol hydrochloride is usually administrated to lung cancer patients through intravenous infusion at a slow rate. For adults and children 12 years and older, 15 mg of ambroxol hydrochloride is given 2 to 3 times daily, while a higher dose (up to 30 mg) might be used in some severe cases. However, a daily dose of up to 1 g of ambroxol hydrochloride has been proven safe and effective in clinical practice (10-12).

In this study, patients were given a 90 mg dose of ambroxol hydrochloride each time, which is considered as a relatively high dose but still within the clinical safe range. Pharmacokinetics of a single dose of ambroxol hydrochloride show that the plasma protein binding rate with the compound is 90%. Ambroxol hydrochloride presents fast tissue distribution targeting the liver. The plasma half-life of the drug is between 7-12 hours. Ambroxol hydrochloride is mainly metabolized in liver through conjugation, and 90% of the metabolite is cleared by kidney (13-15). Generally, most drugs, especially chemotherapeutic agents, only achieve desirable efficacy when they are administered multiple times. After multiple administrations in fixed intervals, higher baseline drug concentrations will build up in the body, and eventually reach a stable state. In the patients who received more than 3 doses (90 mg each time) of ambroxol hydrochloride infusion beginning 48 hours or more before chemotherapy, concentrations of chemotherapy drugs in lung sputum gradually reached a stable state, indicating that the optimal effects of ambroxol hydrochloride could be achieved if it is administered more than 48 hours before chemotherapy treatment.

In previous studies, a paclitaxel/carboplatin combination in the treatment of late stage NSCLC demonstrated response rates of 27% to 40%, with the median survival time of 10 to 12 months and one-year survival rates of 30% to 54% (16). The clinical efficacy of chemotherapy drugs largely rely on the chemosensitivity of tumor cells, and also tightly correlate to the concentration of drugs in the target tissue. Chemotherapy drugs that is attenuated by protein kinase C-delta (PKCδ) (17). It has been reported that PKCδ and its downstream targets play a critical role in mediating inflammation and apoptosis in vascular diseases, such as intimal hyperplasia and aneurysm (18,19). Therefore, inhibition of PKCδ may serve as a strategy to improve efficacy of chemotherapy drugs. In addition, combination therapy, such as using higher doses of paclitaxel and carboplatin, could slow down tumor progression and improve median survival time; however, it could also increase hematologic toxicity, particularly 3/4 grade neutropenia (20). Our findings demonstrate that ambroxol hydrochloride in combination with paclitaxel and carboplatin significantly elevated the drug concentrations in the lung, without increasing the hematologic toxicity, due to the fact that doses per unit body surface area remained unchanged.

In summary, ambroxol hydrochloride can increase chemotherapy drug concentrations in sputum, and optimal effects are achieved when ambroxol hydrochloride administered over 48 hours prior to chemotherapy.

Ambroxol hydrochloride combined with paclitaxel/carboplatin enhances the concentration of these chemotherapeutic agents to an optimal level without introducing hematologic toxicity. Furthermore, follow-up observation studies are required to evaluate whether the combination treatment prolongs progression free survival rates and increases median survival time.

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