MATERIALS AND METHODS

Drugs

Brand name	Manufacturer	Distributor	Preparation	Lot No.
Cisplatin Injection (DBL®)	F H Faulding & Co., Ltd. (Australia)	Indochina Healthcare Ltd. (Thailand)	10 and 50 mg bottle	(B) 8031880
Fosfomycin sodium for injection (Fosmicin®)	Meiji Seika Kaisha (Japan)	Thai Meiji Pharmaceutical Co., Ltd. (Thailand)	2 gm/vial	FOLD 839

Cisplatin and fosfomycin sodium for injection used in this study were donated by the Thai Meiji Pharmaceutical Co., Ltd. Other medication supplies were purchased from the Maharaj Nakhon Chiang Mai Hospital's Pharmacy Service Division.

Subjects

Male patients with pathological diagnosis of NSCLC enrolled in this study were recruited from the Outpatient Department, Division of Oncology, Department of Medicine, Faculty of Medicine, Chiang Mai

University. All selected patients were admitted at the Department of Medicine, Faculty of Medicine, Chiang Mai University.

Inclusion criteria

1. Patients with advanced NSCLC who were cisplatin naïve and were dued to receive cisplatin-based chemotherapy.
2. Age between 18-70 years.
3. Serum BUN < 30 mg/dL, serum creatinine < 2 mg/dL, creatinine clearance > 70 mL/min.
4. Performance status (PS) of 70% or higher on the Karnofsky Scale (see Appendix A).
5. Patients who had concurrently by stable and mild chronic diseases might be included, however, must be discussed on a case-by-case basis prior to screening.
6. All concomitant therapies were approved by the investigator prior to the subject entering the study. Ability to understand the study and comply with the protocol procedures
7. Given written informed consent.

Exclusion criteria

1. Presence of any diseases that affected renal tubular enzyme excretion (e.g., diabetic nephropathy, renal hypertension, urinary tract infection, pyelonephritis, nephrolithiasis, glomerulonephritis, nephrotic syndrome and rheumatic diseases) or any conditions that

increase the risk to the subject revealed by complete medical history, physical examination, and clinical laboratory tests.

2. Hepatic dysfunction (liver function test greater than 3 times the upper limit of normal).
3. Leukocytes count < 3,000/μl, and platelet count < 100,000/μl.
4. Allergic to cisplatin, vinblastine and fosfomycin.
5. Patients receiving nephrotoxic drugs including aminoglycosides, NSAIDs, gold salts, cyclosporine, rifampin and steroids within 1 month prior to the study day.

Study procedures

The protocol of the study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University. Written informed consent was obtained from each subject before initiation of the study. The study was a prospective, randomized open label study. Male patients with NSCLC were given a standard regimen of 100 mg/m^2 cisplatin in combination with 8 mg/m^2 vinblastine on Day 0 of each cycle every 4 weeks. On each cycle, patients were randomly given cisplatin plus vinblastine alone or cisplatin plus vinblastine plus fosfomycin for a maximum of 4 consecutive cycles. Cisplatin reconstituted with 100 mL normal saline solution (0.9% NaCl) was given intravenously over a 2-hour period after saline hydration and mannitol diuresis. Serum magnesium and calcium were monitored and replacement therapy was given as needed. Fosfomycin was given 2 gm (dissolved in 100 mL of 0.9% NaCl) intravenously twice daily for 5

days. The first dose of fosfomycin was given within 2-3 hours before cisplatin administration on Day 0. Thereafter, the second dose was given 12 hours after the first dose. The consecutive doses were given twice daily and continued for a total of 5 days. Concomitant anticancer therapy and prophylactic therapy for nausea and vomiting as well as medication for pain relief (excluding NSAIDs) were permitted. However, these medications should be recorded and administered in identical manner between the two study periods. The patients were discharged on Day 7 after completion of urine collection and returned to the ward 4 days before the starting of the subsequent cycle. All other medications were provided in the same manner on every cycle. Schedule of Time and Events were showed in Appendix B.

Specimens collection

Clinical chemistry and haematology were determined prior to and after cisplatin administration of each cycle. These included blood urea nitrogen (BUN), Na^+, K^+, Cl^-, CO_2, Ca^{2+}, Mg^{2+}, albumin, globulin, ALT, alkaline phosphatase, bilirubin, complete blood count (CBC) and platelet count. Fasting blood samples (5 mL each) for chemistry were collected 2 days before [Day (-2)] and on Day 7 after giving cisplatin. Two milliliters of blood were also collected on [Day (-2)] and Day 5 for CBC and platelets count. The blood samples were sent to the laboratory immediately after collection.

The 24-hour urine were collected on the day before [Day (-1)] and at Day 3, Day 6 after cisplatin administration. Three milliliters of

blood was collected on the morning of Day 0, Day 4, and Day 7 for Scr analysis. After 24 hour collection, pooled urine volume was measured and a 2 mL of urine were aliquoted into three clean plastic vials and kept at -20° C until analysis for NAG activity. The urine creatinine and Scr were determined immediately after collection. All these parameters were used as indicators of nephrotoxicity.

Assay procedure

Urinary NAG

Urinary NAG activity was measured by using a NAG Assay Kit (Shionogi Co. Ltd. Japan). A 50 μL aliquot of the 24-hour urine specimen was added to 12 mL of a prewarmed enzyme reaction mixture containing substrate (Sodio-*m*-cresolsulfonphthaleinyl N-acetyl-β-D-glucosaminide dissolved in 0.05 M sodium citrate buffer, pH 4.9). During incubation at 37°C for 15 min, enzyme hydrolysis of substrate liberated the *m*-cresolsulfonphthaleinate ion. After incubation, the reaction was then terminated by adding 2 mL of 0.3 M sodium bicarbonate buffer, and the reaction product (di-sodium-*m*-cresolsulfonphthaleinate) was measured by using a spectrophotometer at 580 nm. The NAG activity in unit/gram creatinine was reported as the amount excreted/24 hours/gram of creatinine to normalize differences in urinary volume and/or incomplete urine collection [94].

Serum creatinine and creatinine clearance

Serum creatinine and urine creatinine were automatically determined by a chemistry auto-analyzer using Jaffe reaction [14].

Creatinine clearance was calculated according to the formulation below.

$$CL_{cr}\ (mL/min) = \frac{UV}{Scr} X \frac{1.48}{BSA}$$

where U = concentration of urine creatinine (mg/dL)

V = urine volume (mL/min)

Scr = serum creatinine concentration (mg/dL)

BSA = body surface area (m^2)

1.48 = the standard body surface area (m^2)

Statistical Analysis

The values of urine NAG activity, Scr and CL_{cr} of Day 0 were used as pre-cisplatin treatment renal function parameters, while those values of Day 3 and Day 6 were the post-cisplatin treatment parameters. The mean and standard deviation (S.D.) for the sample observations were determined. Thereafter, the test for differences of mean between pre and post-cisplatin therapy (Day 0 Vs Day 3 Vs Day 6) as well as between treatment regimens (cisplatin alone Vs cisplatin plus fosfomycin) were determined. Statistical tests were performed using the STATA program for PC. To compare the differences between the two

groups, the data were first tested whether the observations were drawn from normally distributed populations or not using Shapiro-Wilk-W test. If the distribution of the observations was normally distributed, ANOVA, and *t*-tests would be used to test the hypothesis. However, if the observations yielded the data that were not compatible with normal distribution, the parametric methods would become unreliable because the mean and S.D. could not adequately describe the population and might produce a misleading picture. In such case, the distribution-free or non-parametric Mann-Whitney test would be the best approach to test the hypothesis.