Journal of Toxicology and Environmental Health, Part A, 69:1337–1344, 2006

ISSN: 1528–7394 print / 1087–2620 online
DOI: 10.1080/15287390500360398

ACCUMULATION OF CHROMIUM AND NICKEL METALS IN LUNG TUMORS FROM LUNG CANCER PATIENTS IN TAIWAN

Chung-Yih Kuo[1], Ruey-Hong Wong[1], Jia-Yi Lin[2], Ji-Ching Lai[2], Huei Lee[2]

[1]Department of Public Health, College of Health Care and Management, Chung Shan Medical University, Taichung, Taiwan, Republic of China
[2]Institute of Medical & Molecular Toxicology, College of Medicine, Chung Shan Medical University, Taichung, Taiwan, Republic of China

Metallic carcinogenicity is generally thought to generate of free radicals, and thus some metals were reported to play a role in lung tumorigenesis. In order to verify the role of heavy metals in the development of Taiwanese lung cancer, a case-control study was conducted to compare heavy metal contents between 60 tumor and 42 normal lung tissues surgically resected from lung cancer and noncancer patients. The tissue concentration of heavy metals, including cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), and nickel (Ni), was measured using by atomic absorption spectrometry (AAS). Our results indicated that Cr and Ni contents in lung tumors of lung cancer patients were significantly higher than those in normal lung tissue of noncancer controls, but Co content was markedly lower in lung tumors. Additionally, Pb content in lung tumors was associated with nodal involvement, and Co amounts in squamous-cell carcinomas were relatively higher than those in adenocarcinomas. Data suggest that accumulation of Cr and Ni in lung tumors may play a role, at least in part, in the development of lung cancer in Taiwan.

Lung cancer is the leading cause of cancer mortality in the world, including in Taiwan. The relationship between environmental exposure to heavy metals and increased risk of lung cancer has been explored in a number of epidemiological studies (Sorahan & Lancashire, 1994; 1997; Sorahan et al., 1998). However, data and some of analyses for specific heavy metals with respect to lung cancer are conflicting, and control of factors such as coexposure for other carcinogenic heavy metals occurred in only a few of the studies (Sorahan et al., 1998; Lamm et al., 1992). Previous studies can provide little evidence of an increased risk of lung cancer in humans following prolonged exposed to heavy metals.

Heavy metals interfere with many cellular reactions. The carcinogenic potential of cadmium (Cd), chromium (Cr), cobalt (Co), and nickel (Ni) was reported for humans and experimental animals (Rowbotham et al., 2000; Hayes, 1997; Moulin et al., 1998). One mechanism proposed frequently is an

Received 1 May 2005; accepted 15 July 2005.
Address correspondence to Huei Lee, PhD, Professor and Director, Institute of Medical and Molecular Toxicology, Chung Shan Medical University, No. 110, Sec. 1, Chien-Kuo N. Rd., Taichung, 40203, Taiwan, ROC. E-mail: hl@csmu.edu.tw

increase in oxidative DNA damage attributable to heavy metal exposure, mediated by increased generation of highly reactive oxygen species (ROS) (Shi et al., 1998; Kasprzak & Ward, 1991; Dally & Hartwig, 1997; Kasprzak et al., 1997; Salnikow et al., 2000). Regarding lead (Pb), the epidemiological data are not conclusive with respect to human carcinogenicity, but carcinogenic and cocarcinogeneic effects of lead compounds were suggested in experimental animals (Roy & Rossman, 1992).

It has not yet been established whether altered internal heavy metal concentrations are mainly the cause or the effect of the malignancy. Hence, investigation of heavy metals changes in target tissues might be helpful to interpret the role of essential elements in pulmonary carcinogenesis induction. Heavy metal levels in human circulating blood and urine were used as biological indicators of exposure to heavy metals (Vallyathan et al., 1998; Rafnsson et al., 1997; Hengstler et al., 2003). Available epidemiological studies, however, had no reliable estimates of individual accumulated doses in target tissues, and thus there was limited sensitivity to detect a carcinogenic effect induced by heavy metals. In some cases heavy metal levels were estimated in lung tissues (Anttila et al., 1989; Adachi et al., 1991), but differences in heavy metal levels between cancerous tissue obtained from lung cancer subjects versus normal lung tissue of subject without cancer have not been well examined. Therefore, a case-control study was conducted to evaluate the difference in heavy metal content between lung tumors of lung cancer patients and normal lung tissues from noncancer control subjects.

MATERIALS AND METHODS

Study Subjects and Specimen Collection

Between 1994 and 1998, a hospital-based case-control study was conducted in central Taiwan to elucidate the role of some heavy metals contaminants on the development of lung cancer in Taiwan. Sixty patients with primary lung cancer (International Classification of Diseases, 9th rev.; ICD code 162) were operated on at Taichung Veterans General Hospital in Taichung, Taiwan. Lung tumor samples were surgically resected from lung cancer patients. Cancer cases also underwent a series of examination of pathological stages by board-certified pathologists. Tumor types and stages were determined according to the World Health Organization classification (World Health Organization, 1981). In this study adenocarcinoma was the most common histological type (25 cases, 41.7%), followed by squamous-cell carcinoma (35 cases, 58.3%).

During the same period, 42 potential controls were randomly selected from patients with no history of cancer. They were admitted to one of three teaching medical centers in Taiwan (Veterans General Hospital, Taichung; Chen-Kung University Hospitals, Tainan; and Changhwa Christian Hospital, Changhwa) for treatment or evaluation of different lung diseases, including

pneumothorax, tuberculosis, chest wall deformity, cryptococcal infection, and fibrosis. Similar to the cases of lung cancer in current study, the normal lung tissue for the controls was taken by surgery. Tissue samples for the cases and controls were immediately snap-frozen after excising and were subsequently stored at −80°C until the time of analysis.

None of the study subjects received radiation therapy or chemotherapy prior to surgery. Demographic data were collected on each of these individuals, including age, gender, smoking, and occupational status, from patient interview and a review of the hospital charts with informed consent. The smoking history of a subject included the number of cigarettes smoked daily and also the duration of smoking habit. A parameter termed "pack-years" was coined as an indicator of cumulative smoking dose of a subject and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. This research protocol was approved by the local ethics committee at each study center.

Determination of Heavy Metal Levels

Heavy metals levels in lung tissue, including cadmium, chromium, cobalt, lead, and nickel, were measured using by atomic absorption spectrometry (AAS) with the graphite furnace (Perkin Elmer model 4110ZL) technique and Zeeman background correlation. All analytical glass and plasticware purchased were of low-metal grade and were further cleaned with diluted nitric acid before use. Initially, all of the frozen tissues were equilibrated for 0.5 h at room temperature and then heated for 4 h at 110°C. Subsequently, the dry tissue samples were digested with 2 ml of 65% nitric acid and 1 ml of 30% hydrogen peroxide. After colling, the solutions were diluted to 5 ml with deionized water and stored at −20°C until required for analysis. Palladium was further used as a matrix modifier in the experimental procedure.

For analyses of tissue heavy metals, the accuracy of the instrumental methods and the analytical procedure was checked by using reference solutions (standard reference material [SRM] no. 1566; dogfish reference muscle-2 [DORM-2]; and dogfish liver tissue-2 [DOLT-2]), which were run after every batch of samples. The mean recovery ranged from 90 to 105%, and coefficients of variation (CV) for reproducibility were all less than 10%. Metal concentrations in lung tissue were calculated as micrograms per gram dry weight. Tissue samples were analyzed blind to the clinical status of the individuals for the presence of heavy metals.

Statistical Analysis

Comparisons between the case and control groups for age, gender, and smoking status were made using Student's *t*-test for continuous variables and a χ^2 test for discrete variables. Furthermore, a nonparametric test evaluated the difference in metal contents in different lung tissues, due to skewing of concentration value. A probability of .05 or less was considered as significant.

RESULTS

Characteristics of Lung Cancer Cases and Noncancer controls

The main characteristics of the study subjects investigated in this case-control study are given in Table 1. Among the 60 cases with primary lung cancer, the mean age was 65.1 yr. Table 1 Forty-four (73.3%) of the case subjects were current smokers. Difference in age was statistically significant when comparing the case and control groups. However, cigarette smoking and gender were not significantly different when comparing these two groups.

Comparison of Metal Levels Between Lung Cancer Patients and Noncancer Controls

The heavy metal contents in lung tissue of study subjects are summarized in Table 2. Lung cancer cases were observed to have significantly elevated levels of Cr and Ni in tumor tissue than those in normal lung tissue of noncancer controls. Inversely, a significantly lower level of Co was found in lung cancer cases than that in noncancer controls. In lung tumors, the highest amount of heavy metal contaminant was Cr, followed by Pb, Ni, Cd, and Co. Subsequently, to understand the contribution of cigarette smoking to heavy metal accumulation in lung tumors, the median value of individual cigarette consumption (60 pack-years) was used as a reference to categorize smoking lung cancer cases into mild and heavy smoking groups. It was found that Cr content in mild smoking cases was significantly higher than in heavy smoking cases (Table 3).

TABLE 1. Characteristics of Lung Cancer Cased and Noncancer Controls

Variables	Lung cancer cases (%)	Noncancer controls (%)
Number	60	42
Age	65.1 ± 1.1[a,b]	51.9 ± 2.7
Gender		
Female	11 (18.3)	8 (19.0)
Male	49 (81.7)	34 (81.0)
Smoking status		
Yes	44 (73.3)	24 (57.1)
No	16 (26.7)	18 (42.9)
Tumor type		
Adenocarcinoma	25 (41.7)	
Squamous-cell carcinoma	35 (58.3)	
Tumor stage		
I	25 (41.7)	
II	14 (23.3)	
III	21 (35.0)	

[a]Results were given as mean ± SE.
[b]Significant difference from control ($p < .05$).

TABLE 2. Comparison of Metal Levels (μg/g) Between Lung Cancer Patients and Noncancer Controls

Metals	Lung cancer cases[a] (n = 60)	Noncancer controls (n = 42)
Cadmium	0.48 ± 0.06[b]	0.70 ± 0.13[b]
Chromium	5.46 ± 1.59[b,d]	4.68 ± 1.30[b]
Cobalt[c]	0.18 ± 0.03[b,d]	0.25 ± 0.06[b]
Lead	2.41 ± 0.50[b]	2.82 ± 0.92[b]
Nickel	1.77 ± 0.60[b,d]	1.26 ± 0.27[b]

[a]Compared to the metal in normal lung tissue of controls.
[b]Results were given as mean ± SE.
[c]Fifty-seven cases were available for Co analysis.
[d]Significant difference from control ($p < .05$).

TABLE 3. Differences in Metal Levels (μg/g) Between Mild and Heavy Smokers in Lung Cancer Patients

Metal	Light smoker (n = 17)	Heavy smoker (n = 21)
Cd	0.50 ± 0.12	0.56 ± 0.10
Cr	8.66 ± 3.51	1.70 ± 0.19[a]
Co	0.11 ± 0.01	0.26 ± 0.07
Pb	1.54 ± 0.15	3.77 ± 1.38
Ni	4.13 ± 1.99	0.82 ± 0.15

Note. Thirty-eight lung cancer cases were available for the categories by cigarette smoking consumption. The median value of cigarette consumption (60 pack-years) was used as reference value to categorize smoking lung cancer cases into mild and heavy smoking groups.
[a]Significant difference from light smoker ($p < .05$).

Differences in Metal Levels Between Mild and Heavy Smokers Among Lung Cancer Patients

The association of heavy metals in lung tumor tissues with the clinicopathological parameters of lung cancer patients was also statistically analyzed, as shown in Table 4. Among the clinicopathological parameters, including age, gender, tumor type, tumor stage, smoking status, and T and N values, only nodal involvement was significantly associated with Pb content.

DISCUSSION

Chromium, a ubiquitous environmental and industrial contaminant, is a well-known human carcinogen, particularly in human lung cancer (Sorahan et al., 1998; Hayes, 1997; Anttila et al., 1989). Chromium was found to be a potential inducer of tumors in experimental animals, and neoplastically transformed

TABLE 4. Relationship Between Metal Levels (μg/g) and Clinicopathological Parameters in Lung Cancer Patients.

Parameters	Case Number	Cd, mean ± SE	Cr, mean ± SE	Co,[a] mean ± SE	Pb, mean ± SE	Ni, mean ± SE
Age						
<65 yr	26	0.42 ± 0.07	5.79 ± 2.54	0.20 ± 0.05	2.15 ± 0.58	1.84 ± 1.00
≥65 yr	34	0.51 ± 0.09	5.20 ± 2.05	0.17 ± 0.03	2.60 ± 0.77	1.71 ± 0.73
Gender						
Female	11	0.38 ± 0.10	5.29 ± 4.01	0.18 ± 0.04	1.95 ± 0.34	1.15 ± 0.55
Male	49	0.49 ± 0.07	5.49 ± 1.74	0.18 ± 0.03	2.51 ± 0.60	1.90 ± 0.71
Tumor type						
AD	25	0.36 ± 0.06	6.72 ± 3.11	0.11 ± 0.01	2.12 ± 0.60	1.67 ± 0.86
SQ	35	0.55 ± 0.09	4.56 ± 1.60	0.23 ± 0.04	2.61 ± 0.74	1.85 ± 0.82
T value						
T1/T2	44	0.48 ± 0.07	6.89 ± 2.13	0.19 ± 0.04	2.81 ± 0.68	2.22 ± 0.80
T3/T4	16	0.47 ± 0.11	1.54 ± 0.28	0.17 ± 0.08	1.32 ± 0.03	0.54 ± 0.07
N value						
N0	29	0.49 ± 0.10	7.82 ± 2.87	0.22 ± 0.06	3.39 ± 1.01	2.68 ± 1.20
N1/N2	31	0.46 ± 0.08	3.26 ± 1.46	0.15 ± 0.02	1.50 ± 0.11[b]	0.92 ± 0.22
Stage						
I/II	39	0.46 ± 0.07	7.32 ± 2.38	0.20 ± 0.04	2.94 ± 0.75	2.40 ± 0.90
III	21	0.49 ± 0.09	2.00 ± 0.55	0.14 ± 0.02	1.43 ± 0.09	0.49 ± 0.06
Smoking status						
Yes	44	0.52 ± 0.07	5.93 ± 1.93	0.18 ± 0.03	2.58 ± 0.67	2.05 ± 0.79
No	16	0.34 ± 0.09	4.15 ± 2.75	0.17 ± 0.03	1.93 ± 0.28	0.99 ± 0.38

Note. AD, Adenocarcinoma; SQ, squamous-cell carcinoma; T value, extent of primary turnor; N value, regional lymph node involvement.

[a]Fifty-seven lung cancer cases were available for Co analysis.

[b]Significant difference from N0 ($p < .05$).

cells in culture (Singh et al., 1998). Chromium accumulation in lung tissues was found in workers with occupational exposure to Cr and cigarette smoking (Paakko et al., 1989), and workers with occupational exposure to Cr and cigarette smoking (Paakko et al., 1989), and workers with occupational exposure to Cr have a higher incidence of lung cancer as shown in Rowbotham et al. (2000) and Sorahan et al. (1998), in which it was suggested that the Cr contaminant is involved in human lung tumorigenesis. Nickel is also a ubiquitous environmental and industrial contaminant. It is widely used in industrial processes, such as electroplating and the manufacture of steel and batteries (Frenkel et al., 1994; Kiilunen et al., 1997; Horng et al., 2003). Epidemiological studies showed that occupational exposure to Ni is associated with a high incidence of human lung and nasal cancers (Andersen et al., 1996; Grimsurd et al., 2003). Nickel can also produce cell transformation and induce tumors in animal models (Salnikow et al., 1999; Kasprzak et al., 2003). In our study, cigarette smoking consumption may not explain the accumulation of Cr and Ni in lung tumors because Cr and Ni in light smokers were present at higher levels than in heavy smokers (Table 3). Thus, in addition to cigarette smoking, data

suggest that environmental/occupational exposure of Cr and Ni may be important determinants in Taiwanese lung cancer, although no occupational data were available in our study.

Cobalt is genotoxic in vitro and in vivo, and carcinogenic in rodents (De Boeck et al., 2003). Some epidemiological data showed that occupational exposure of Co was linked to an increased lung cancer risk (Moulin et al., 1998; Tuchsen et al., 1996), but further studies should be undertaken before firm conclusions are drawn. In our study, the Co content in lung tumors was found at a significantly lower level than in noncancer controls.

Lead is not genotoxic in vitro, but increases the mutagenicity of other mutagens, possibly acting via inhibition of DNA repair (Hengstler et al., 2003). Early animal studies indicated that Pb does induce lung cancer in animal studies (Shimkin et al., 1977; Poirier et al., 1984), and IARC deemed these animal studies sufficient for demonstrating animal carcinogenesis of Pb. Thus, Pb was classified by IARC as a possible human carcinogen. No difference in Pb levels was found in our case-control study.

In our case-control study, data indicated that higher Cr and Ni accumulation occurred in lung cancer patients compared noncancer controls, and may contribute to lung tumorigenesis in Taiwanese lung cancer patients.

REFERENCES

Adachi, E., Hayashi, T., and Hashimoto, P. 1991. A comparison of the immunofluorescent localization of collagen types I, III, and V with the distribution of reticular fibers on the same liver sections of the snow monkey *Macaca fuscata*. *Cell Tissue Res*. 4:1–8.

Andersen, A., Berge, S. R., Engeland, A., and Norseth, T. 1996. Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. *Occup. Environ. Med*. 53:708–713.

Anttila, S., Kokkonen, P., Paakko, P., Rainio, P., Kalliomaki, P. L., Pallon, J., Malmqvist, K., Pakarinen, P., Nanto, V., and Sutinen, S. 1989. High concentrations of chromium in lung tissue from lung cancer patients. *Cancer* 63:467–473.

Dally, H., and Hartwig, A. 1997. Induction and repair inhibition of oxidative DNA damage by nickel II and cadmium II in mammalian cells. *Carcinogenesis* 18:1021–1026.

De Boeck, M., Hoet, P., Lombaert, N., Nemery, B., Kirsch-Volders, M., and Lison, D. 2003. In vivo genotoxicity of hard metal dust: Induction of micronuclei in rat type II epithelial lung cells. *Carcinogenesis* 24:1793–1800.

Frenkel, K., Karkoszka, J., Cohen, B., Baranski, B., Jakubowski, M., Cosma, G., Taioli, E., and Toniolo, P. 1994. Occupational exposures to Cd, Ni, and Cr modulate titers of antioxidized DNA base autoantibodies. *Occup. Environ. Med*. 102(suppl. 3): 221–225.

Grimsrud, T. K., Berge, S. R., Haldorsen, T., and Andersen, A. 2003. Exposure to different forms of nickel and risk of lung cancer. *Am. J. Epidemiol*. 156:1123–1132.

Hayers, R. B. 1997. The carcinogenicity of metals in humans. *Cancer Causes Control* 8:371–385.

Hengstler, J. G., Bolm-Audorff, U., Faldum, A., Janssen, K., Reifenrath, M., Gotte, W., Jung, D., Mayer-Popken, O., Fuchs, J., Gebhard, S., Bienfait, H. G., Schlink, K., Dietrich, C., Faust, D., Epe, B., and Oesch, F. 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis* 24:63–73.

Horng, C. J., Horng, P. H., Hsu, J. W., and Tsai, J. L. 2003. Simultaneous determination of urinary cadmium, cobalt, lead, and nickel concentrations in steel production workers by differential pulse stripping voltammetry. *Arch. Environ. Health* 58:104–110.

Kasprzak, K. S., and Ward, J. M. 1991. Prevention of nickel subsulfide carcinogenesis by local administration of *Mycobacterium bovis* antigen in male F344/NCr rats. *Toxicology* 679:97–105.

Kasprzak, K. S., Jaruga, P., Zastawny, T. H., North, S. L., Riggs, C. W., Olinski, R., and Dizdaroglu, M. 1997. Oxidative DNA base damage and its repair in kidneys and livers of nickel II-treated male F344 rats. *Carcinogenesis* 18:271–277.

Kasprzak, K. S., Sunderman, F. W., Jr., and Salnikow, K. 2003. Nickel carcinogenesis. *Mutat. Res*. 533:67–97.

Kiilunen, M., Aitio, A., and Tossavainen, A. 1997. Occupational exposure to nickel salts in electrolytic plating. *Ann. Occup. Hyg*. 41:89–200.

Lamm, S. H., Parkinson, M., Anderson, M., and Taylor, W. 1992. Determinants of lung cancer risk among cadmium-exposed workers. *Ann. Epidemiol*. 2:195–211.

Moulin, J. J., Wild, P., Romazini, S., Lasfargues, G., Peltier, A., Bozec, C., Deguerry, P., Pellet, F., and Perdrix, A. 1998. Lung cancer risk in hard-metal workers. *Am. J. Epidemiol*. 148:241–248.

Paakko, P., Kokkonen, P., Anttila, S., and Kalliomaki, P. L. 1989. Cadmium and chromium as markers of smoking in human lung tissue. *Environ. Res*. 49:197–207.

Poirier, L. A., Theiss, J. C., Arnold, L. J., and Shimkin, M. B. 1984. Inhibition by magnesium and calcium acetates of lead subacetate- and nickel acetate-induced lung tumors in strain A mice. *Cancer Res*. 44:1520–1522.

Rafnsson, V., Gunnarsdottir, H., and Kiilunen, M. 1997. Risk of lung cancer among masons in Iceland. *Occup. Environ. Med*. 54:184–188.

Rowbotham, A. L., Levy, L. S., and Shuker, L. K. 2000. Chromium in the environment: An evaluation of exposure of the UK general population and possible adverse health effects. *J. Toxicol. Environ Health B* 3:145–178.

Roy, N. K., and Rossman, T. G. 1992. Mutagenesis and comutagenesis by lead compounds. *Mutat. Res*. 298:97–103.

Salnikow, K., An, W. G., Melillo, G., Blagosklonny, M. V., and Costa, M. 1999. Nickel-induced transformation shifts the balance between HIF-1 and p53 transcription factors. *Carcinogenesis* 20:1819–1823.

Salnikow, K., Su, W., Blagosklonny, M. V., and Costa, M. 2000. Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species-independent mechanism. *Cancer Res*. 60:3375–3378.

Shimkin, M. B., Stoner, G. D., and Theiss, J. C. 1977. Lung tumor response in mice to metals and metal salts. *Adv. Exp. Med. Biol*. 91:85–91.

Shi, X., Castranova, V., Halliwell, B., and Vallyathan, V. 1998. Reactive oxygen species and silica-induced carcinogenesis. *J. Toxicol. Environ. Health B* 1:181–197.

Singh, J., McLean, J. A., Pritchard, D. E., Montaser, A., and Patierno, S. R. 1998. Sensitive quantitation of chromium–DNA adducts by inductively coupled plasma mass spectrometry with a direct injection high-efficiency nebulizer. *Toxicol. Sci*. 46:260–265.

Sorahan, T., Burges, D. C., Hamilton, L., and Harrington, J. M. 1998. Lung cancer mortality in nickel/chromium platers, 1946–95. *Occup. Environ. Med*. 55:236–242.

Sorahan, T., and Lancashire. 1994. Lung cancer findings from the NIOS study of United States cadmium recovery workers: A cautionary note. *Occup. Environ. Med*. 51(2):139–140.

Tuchsen, F., Jensen, M. V., Villadsen, E., and Lynge, E. 1996. Incidence of lung cancer among cobalt-exposed women. *Scand. J. Environ. Health* 22:444–450.

Vallyathan, V., Green, F., Ducatman, B., and Schulte, P. 1998. Roles of epidemiology, pathology, molecular biology, and biomarkers in the investigation of occupational lung cancer. *J. Toxicol. Environ. Health B* 1:91–116

World Health Organization. 1981. *Histological typing of lung tumors*, 2nd ed. Geneva: WHO.