Former Poison Gas Workers and Cancer: Incidence and Inhibition of Tumor Formation by Treatment with Biological Response Modifier N-CWS

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Mustard gas is known to have mutagenic and carcinogenic effects on animal and human cells. In this report, 1,632 male Japanese who worked in poison gas factories at some time between the years 1927 and 1945 were studied to determine comparative risk for development of cancer, the reference population being data on Japanese males overall. The standardized mortality ratio (SMR) for lung cancer in workers directly and indirectly involved in the production of mustard gas was significantly elevated. In addition, SMR for lung cancer in workers who had worked for more than 5 years was also significantly elevated. Thus, poison gas workers who had engaged in the production of mustard gas or related work for more than 5 years are a high-risk group for lung cancer. Under the cancer preventive program, *Nocardia rubra* cell-wall skeleton (N-CWS) was administered to 146 former poison gas workers. During a 4.5-year observation period, development of cancers was found in 7 treated workers and 17 untreated controls. After elimination of the influence of smoking level, a significant suppression of development of cancers was noted in the N-CWS-treated workers as compared to the untreated controls. Although the molecular mechanisms of carcinogenesis in former poison gas workers remains unclear, our study proposes the possible effect of biological response modifiers in the prevention of cancer development in high-risk human subjects. — Environ Health Perspect 104(Suppl 3):485–488 (1996)

Key words: former poison gas workers, mustard gas, lung cancer, *Nocardia rubra* cell-wall skeleton, cancer prevention

Introduction

The defunct Japanese Army had operated a poison gas factory from 1927 to 1945 on Okunojima, an island off the coast of Tadanoumi-cho, Takehara City, Hiroshima Prefecture, Japan. Production of the poison gas reached its peak in 1937. After the war the factory was closed and the remaining poison gases were disposed of in the sea. Table 1 shows the chemical properties of these poison gases. Mustard gas (yperite)

and chlorvinylarsine (lewisite) are erosive gases with high toxicity, diphenylcyan-arsine is a sneezing gas, hydrocyanic gas and phosgene are asphyxiating gases, and chloracetophenone is a tear gas.

The former poison gas workers were divided into three groups according to their type of work in the factory. Group A consisted of workers engaged directly in the production of poison gases such as mustard gas and lewisite. Group B consisted of workers who had come in contact with these gases in laboratories or during repair or inspection in the factory. Group C consisted of those engaged in the production of other gases or who were working in medical or administrative work.

Since 1952, we have been conducting clinical and pathological studies on respiratory tract cancer among these former workers of the Okunojima poison gas factory (1). Wada et al. (2) reported a high incidence

of respiratory neoplasia among poison gas workers in 1968. The present report describes the results of epidemiological and pathological studies on subjects who developed cancer between 1952 and 1992. Furthermore, using the biological response modifier *Nocardia rubra* cell-wall skeleton (N-CWS), the feasibility of preventing cancer development in the former poison gas workers was examined and valuable results were obtained.

Materials and Methods

Subjects who formerly worked in the poison gas factory were identified in several ways. Known workers were asked about the identity of co-workers, persons admitted to local hospitals were questioned about employment at the factory, questionnaires were distributed in a house-to-house canvass of the area where most former factory workers lived, and spot television announcements urged former factory employees to contact the investigators. In 1952, 1,632 poison gas workers had been identified, all of them male. We have been observing the clinical status of the survey group since 1952. Some of the survey group came to the investigators as patients because of their respiratory symptoms, and identified poison gas workers were interviewed at least once a year. When the malignant neoplasms were diagnosed in any hospital, we were notified by the hospital or public-health authorities. The costs of therapy for malignant tumors in poison gas workers were partly paid by Japanese government or Hiroshima Prefecture. A complete record of all these workers has been maintained by our department. Among the subjects, 674 belonged to group A (engaged in production of poison gases), 598 to group B (in contact with gases through laboratories or during repair or inspection), and 360 to

Table 1. Gases produced at the poison gas factory on Okunojima island.

Name of gas	Chemical structure	Character
ββ′-Dichlordiethyl sulfide (mustard gas, yperite)	$(ClCH_2CH_2)_2S$	Erosive
Chlorvinylarsine (lewisite)	$CHCl:CHAsCl_2$ $(CHCl:CH)_2AsCl$ $(CHCl:CH)_3As$	Erosive
Diphenylcyanarsine	$(C_6H_5)_2AsCN$	Sneezing
Hydrocyanic acid	HCN	Asphyxiation
Chloracetophenone	$C_6H_5COCH_2Cl$	Tear gas
Phosgene	$COCl_2$	Asphyxiation

group C (engaged in production of other gases or in medical or administrative work). The number of malignant neoplasms from 1952 to 1992 in 1,632 poison gas workers was surveyed including histological types of lung cancer for specimens obtained from 85 cases by biopsy or autopsy.

To examine the relative risk of the poison gas workers for cancer as compared with the normal population, the standardized mortality ratio (SMR) of these workers due to malignant neoplasms between 1952 and 1992 was calculated. Observed cases were identified by clinical information and death certificates and expected cases were determined from all Japanese national mortality data. SMR was shown as the ratio of observed case number/expected case number. The Student's t-test was employed for all statistical analyses.

To examine the possibility of immunological prevention of cancer development in humans by administering N-CWS, 146 workers, including 80 workers from group A and 66 from group B, were allocated to either the N-CWS treatment group or the untreated control group. The sex, age, type of work in the poison gas factory, and the duration of work were matched between the N-CWS group and the control group. Patients in the N-CWS group received an intradermal administration of 200 µg N-CWS once a month for 4.5 years with informed consent. During the 4.5-year observation period, we compared the incidence of malignant tumors between the two groups.

To remove the influence of confounding variables such as difference of smoking level, age, duration of employment, etc., we employed logistic regression as a method of multivariate regression analysis. Statistical significance of the difference was tested by a one-sided test.

Results

Characteristics of Malignant Neoplasms from 1952 to 1992 in Poison Gas Workers

As shown in Table 2, the total number of patients who developed malignant tumors between 1952 and 1992 was 352 (21.6%), with the occurrence of malignant tumors of the respiratory tract being 8.3%. At the site of development of lung cancer, there was a predominance of the central type in the pulmonary hilus, and the incidence was higher in those who were directly exposed to the poison gases (group A). Of these cases, there were 25 cases of multiple cancers

Table 2. Number of malignant neoplasms in former poison gas workers from 1952 to 1992.[a]

Location of neoplasms	Group A	Group B	Group C	Total
Respiratory system	72 (10.7)[b]	51 (8.5)	13 (3.6)	136 (8.3)
Gastrointestinal tract	85 (12.6)	62 (10.4)	37 (10.3)	184 (11.3)
Others	8 (1.2)	18 (3.0)	6 (1.7)	32 (2.0)
Total	165 (24.5)	131 (21.9)	56 (15.6)	352 (21.6)

Group A workers were involved in production of poison gases; group B workers were in contact with gases through laboratories or during repair or inspection; group C workers were engaged in production of other gases or in medical or administrative work. [a]These data include both dead and living cases and double cancers. [b]Percentage against total number of survivors in 1952 (group A, 674; group B, 598; group C, 360; total, 1632) in parentheses.

Table 3. Histological types of lung cancer in former poison gas workers by group from 1952 to 1992.

Group	Number of cases	Squamous cell carcinoma	Undifferentiated carcinoma Large	Small	Adenocarcinoma	Combined squamous and adenocarcinoma
A	48[a]	26[a]	4	11[a]	8	1
B	27	12	3	8	2	2
C	10	5	0	2	3	0
Total	85[a]	43[a]	7	21[a] (total 28[a])	13	3

Group A workers were involved in production of poison gases; group B workers were in contact with gases through laboratories or during repair or inspection; group C workers were engaged in production of other gases or in medical or administrative work. [a]Includes two cases with two separate types of cancers.

including 15 cases of lung cancer and 2 cases of laryngeal cancer associated with malignant tumors of other organs.

The histological type (Table 3) was confirmed by autopsy or biopsy in 85 cases of lung cancer. Squamous cell carcinoma was found in 43 cases (51%) and undifferentiated carcinoma in 28 cases (33%), while there were fewer cases with adenocarcinoma (15%). Squamous cell carcinoma often occurred in the paranasal sinus, tongue, pharynx, and larynx. Undifferentiated carcinoma and squamous cell carcinoma often occurred in the trachea and central portion of bronchi. These findings indicate that cancers more often occurred at those sites which had been more intensely exposed to mustard gas.

Standardized Mortality Ratio of Malignant Tumors in Poison Gas Workers Compared with Japanese Males Overall

The SMR was calculated from the number of poison gas workers who died of malignancies between 1952 and 1992 and from the national statistics of deaths due to malignancy of individual organs during the same period. Both the death rate due to total malignancies and the death rate due to lung cancers were significantly higher in poison gas workers compared to the national averages. This difference was
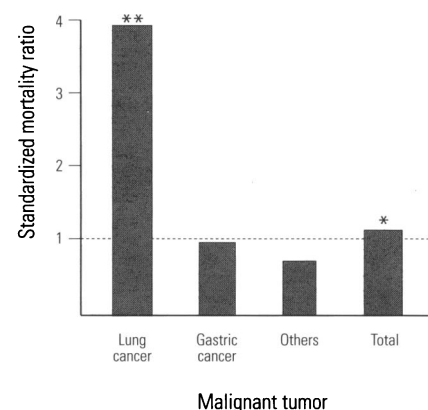

Figure 1. Standardized mortality ratio of malignant tumors in former poison gas workers who died between 1952 and 1992 as compared with Japanese males overall. Both death rates due to total malignancies ($p<0.01$) and lung cancers ($p<0.001$) were significantly higher in poison gas workers. *$p<0.01$. **$p<0.001$.

particularly marked for deaths due to lung cancers (Figure 1).

In evaluating the influence of mustard gas exposure, not only the type of work but also duration of work seems to serve as a significant factor. For this reason, we subdivided each of the three types of work into three groups depending on the duration of work: less than 6 months, from 6 months to 5 years, and more than 5 years.

Table 4. Standardized mortality ratio (SMR) of total malignant neoplasia and lung cancer by group and duration of work from 1952 to 1992.

Group	Duration of work		
	≤0.5 Years	0.5 to 5 Years	5 Years +
Total malignant neoplasias			
A	0.78[a]	1.44*	2.36**
B	1.56	1.23	1.66*
C	0.36	0.9	0.75
Lung cancer			
A	2.32	3.24**	7.35**
B	3.84	2.53	4.92**
C	0	1.08	1.5

Group A, workers were involved in production of poison gases; group B workers were in contact with gases through laboratories or during repair or inspection; group C workers were engaged in production of other gases or in medical or administrative work. [a]SMR = number of observed cases/number of expected cases. *$p < 0.05$. **$p < 0.001$.

Table 5. Development of primary cancers in *Nocardia rubra* cell-wall skeleton (N-CWS)-treated workers and untreated controls.

Cancer	N-CWS-treated workers[a]	Untreated controls[b]
Respiratory tract		
Lung	1	3
Larynx	0	2
Total	1	5
Other organs		
Esophagus	0	1
Stomach	1	4
Colon	0	2
Rectum	1	1
Liver	1	0
Bile duct	1	1
Kidney	1	0
Bladder	0	1
Prostate	0	1
Skin	0	1
Malignant lymphoma	1	0
Total	6	12
Total cancers	7	17*

[a]N-CWS-treated workers: 146 (group A and B) workers with intradermal injection of 200 µg of N-CWS each month. [b]Untreated controls: 146 (group A and B) workers without N-CWS treatment. *$p < 0.05$ (x^2 test).
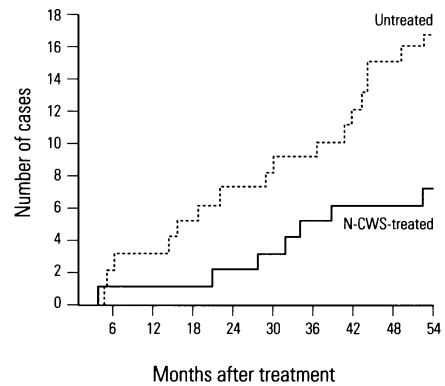

Figure 2. Occurrence of cancers in N-CWS-treated former poison gas workers and untreated controls. The incidence of cancer development during 4.5-year observation period in N-CWS-treated workers was significantly lower than that in untreated workers ($p < 0.05$, x^2 test. $n = 146$ for each group).

In this way, the workers were divided into nine groups, depending on the type and duration of work.

As shown in Table 4, the SMR for total malignancies increased when the work was more involved in exposure to mustard gas and as the duration of work became longer. A similar analysis for lung cancers shows more marked results.

Effect of *Nocardia Rubra* Cell-wall Skeleton on Cancer Prevention in Former Poison Gas Workers

During the 4.5-year observation period, the development of cancers in former poison gas workers was noted in 7 subjects from the N-CWS-treated group and 17 subjects from the untreated control group. Thus, the incidence of cancer development was significantly lower in the N-CWS group ($p < 0.05$). Among them, respiratory cancer occurred in only 1 subject in the N-CWS group while it developed in 5 of the control group (Table 5). Figure 2 chronologically traces the development of cancers during the 4.5-year observation period in each group. In the N-CWS group, the number of subjects who developed cancer became significantly lower than that in the untreated group over time. This result indicates that, when monthly administration of N-CWS (200 µg) was continued, the incidence of clinically detectable cancers among former workers of poison gas factories could be significantly reduced.

Discussion

Animal experiments have demonstrated that mustard gas is a highly mutagenic

carcinogen (*3,4*). A high incidence of cancer of the respiratory tract was reported among the Allied troops exposed to the gas during World War I (*5*).

Cancer of the respiratory tract in poison gas workers was regarded as an occupational cancer that developed even after cessation of exposure to poison gas. The cancer in these workers developed from the upper respiratory tract including the paranasal sinus and the pulmonary hilus. Histologically, squamous cell carcinoma and undifferentiated carcinoma were the predominant types, whereas adenocarcinoma was rare. According to a histological study of 4,931 cases of lung cancer in Japan by Yoshimura and Yamashita (*6*), squamous cell carcinoma accounted for 40.4%, adenocarcinoma for 37.6%, and undifferentiated carcinoma for 18.4%.

The poison gas workers in our study are considered to have inhaled a small amount of the gas over an extended period. The 1,632 workers who were employed in the factory were found to be a high-risk group for lung cancer. The SMR was significantly elevated in groups A and B whose workers had inhaled mustard gas for a period of more than 6 months. The results indicate that lung cancer is induced by mustard gas.

These epidemiological data are in agreement with the results of our previous study of somatic mutation in peripheral lymphocytes (*7*). In that study, the mutation

frequency of hypoxanthine guanine phosphoribosyltransferase was significantly higher in groups A and B and in those who had worked at the poison gas factory for more than 5 years than in the healthy control group (*7*). These results suggest that inhalation of small amounts of mustard gas may damage somatic cell genes.

Immunological abnormalities such as depressed mitogen response and T-cell subset abnormalities have been reported in poison gas workers (*8–12*). Considering the importance of early clinical detection of cancer, these workers have been examined regularly in an attempt to develop useful tumor markers (*13*). In addition, we have attempted to prevent cancer development by immunological means. N-CWS was developed by Azuma et al. (*14*), and the animal experiments conducted by Hirao et al. (*15*) have shown that it prevents cancer development. In April 1984 to prevent cancer development, 146 patients who were volunteers among the former poison gas workers were intradermally administered with N-CWS. It was reported that N-CWS is capable of normalizing depressed immunocompetence and cancer prevention in poison gas workers (*10–12,16*). During the 4.5-year observation period, the incidence of cancer development in the N-CWS-treated workers was significantly lower than that in untreated controls, as analyzed by a simple x^2 test. This result was also confirmed by multivariate regression analysis, removing the influence of age, duration of employment, and difference of smoking level ($p = 0.045$, data not shown).

Thus, it was concluded that monthly administration of N-CWS could prevent cancer development in workers engaged in the production of sulfur mustard. These results suggest a new concept in medicine: the prevention of cancer development using a biological response modifier in humans.

However, the molecular mechanism with which mustard gas exposure leads to excess risk of lung cancer is still unclear. If genetic damage of particular genes contributed to the development of lung cancer in mustard gas workers, the genetic changes may be those frequently observed in small cell carcinoma, large cell carcinoma, or squamous cell carcinoma. In the general population, amplification of the L-*myc* gene, mutation or loss of heterozygosity (LOH) of the p53 and retinoblastoma (*Rb*) genes, and allelic loss of the short arm of chromosome 3 are more frequently observed in small cell carcinoma than in

non-small cell carcinoma (*17*). In this context, we investigated the alteration of these oncogenes and tumor suppressor genes in three lung cancer tissues recently obtained from former poison gas workers, as well as lung cancers in the general population. One of these three cases showed mutation and LOH of the p53 gene, one case showed mutation only, and the remaining case showed no alterations. These results are not unique compared to other lung cancer cases or reports of other investigators. In addition, LOH of the *Rb* gene, point mutations of the K-*ras* gene, and amplification of the L-*myc* gene were not particular in these cases [Cases F91-103, B5, and F90-22 in Hiyama et al. (*18*)]. Takeshima et al. (*19*) reported mutations in the p53 gene in 12 lung carcinomas from mustard gas workers and found eight mutations in six cases. There were two cases with double mutations,

which is a rare phenomenon in the general population. However, since the mutational spectra and frequency were similar to other reports of lung cancer in the general population, it is difficult to elucidate the relationship between the alteration of the p53 gene and mustard gas exposure.

What factors contributed to the excess risk of lung cancer among the poisoned gas exposed group? There are several possible explanations: there was a specific damage on an unknown regulatory gene; mutation rates of various genes were elevated non-specifically; or an increase of cell division caused by chronic inflammation may have contributed to an elevation in the incidence of mutations. Further genetic investigations on lung cancers that developed early after mustard gas exposure is necessary to elucidate the mechanism of carcinogenesis in poison gas workers.

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