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Epidemiology of Biliary Tract Cancer in Japan: Analytical Studies

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Summary. **Epidemiological studies of biliary tract cancer (BTC) have been conducted over the last decade, the rationale being that the death rates in Niigata Prefecture where we live were found to be the highest in Japan for this cancer. The present paper proposes a hypothesis for seeking a determinant factor(s) and test this by conducting analytical studies.**

Since it was suggested that the prefectures in Japan and the cities and counties in Niigata where the mortalities from BTC were higher corresponded with rice producing areas, we began to search for a link between rice production and BTC mortality. Environmental factors taken into consideration included agricultural chemicals, dietary patterns which may be specific to rice producing areas and geological characteristics of the soil and drinking water. None of these factors, however, seems to have acted as a single cause in the etiology of BTC.

On the basis of our analytical studies, we generated a multifactorial causation hypothesis: those who have a genetic susceptibility and a past history of gallstones or cholecystitis risk suffering from BTC (female gallbladder cancer in particular) when they are exposed to geographically specific environmental factors such as agricultural chemicals (e.g. diphenylether herbicides and their metabolites) in paddy fields. Based on the gas-chromatographic analysis of tap water, the waterborne route via the contaminated Shinano and Agano rivers is the most suspicious.

INTRODUCTION

Epidemiological studies of biliary tract cancer (BTC) have been conducted for the last decade. The rational reason for this was that BTC death rates in Niigata Prefecture where we live were found to be the highest in Japan.[1]

Although BTC, including gallbladder cancer (GBC) and extrahepatic bile duct cancer (BDC), has still received less attention in epidemiological studies in Japan, its study comprises an intriguing field of epidemiology, since its geographical distribution of death rates shows a characteristic clustering pattern.[1-3]

In the present paper our main purpose is to propose a hypothesis for seeking a determinant factor or factors, and to test this epidemiologically by conducting analytical studies. Our research experiences concerning the formulation of a working hypothesis and analytical studies are presented.

DESCRIPTIVE STUDIES

A clustering of deaths from BTC has been noted in the prefectures in the northeastern parts of Japan.[1-3] Among these prefectures the death rates of BTC in both sexes in Niigata have been the highest since the late 1960s. A demographic analysis revealed that the death cluster corresponded with the Niigata plains and Sado island while no cluster was observed in the other parts of Niigata.[2] A recent analysis of BTC revealed clustering owing to the occurrence of GBC[4] (female GBC in particular[5]). In addition, the SMRs of GBC in Murakami, Gosen and Kamo Cities were found to be not as high, even though these lie in the midst of the Niigata plains. As we will mention later in the present paper, the major sources for drinking water were from groundwater in both Murakami and Gosen Cities, and partly from the Kamo river, originating in mountainous areas, in Kamo City. The details of the descriptive epidemiology are reported elsewhere.[5]

MODEL BUILDING AND FORMULATION OF HYPOTHESIS

The descriptive epidemiology of BTC in Japan and Niigata Prefecture showed that the prefectures in Japan and the cities and counties in Niigata where the mortality from BTC was highly corresponded with rice producing areas.[2] In fact, an ecological correlation analysis between the amounts of rice production per area of rice field in 47 prefectures in 1975 and their SMRs of BTC in the same year revealed a positive association: $r=0.544$ ($p<0.001$) in males and $r=0.377$ ($p<0.01$) in females. The statistically significant correlation was also observed between the amounts in 1970 and the SMRs in 1975: $r=0.567$ ($p<0.001$) in males and $r=0.440$ ($p<0.001$) in females.

If such ecological correlation should actually exist, it ought to prove worthwhile to find a link between rice production and the mortality of BTC. We tried to build a model in order to explain how they are related to each other. As a possible link between them, some environmental factors were taken into consideration, for example, agricultural chemicals, dietary patterns which may be specific to rice producing areas, geological characteristics such as trace elements, and mutagenic substances in the environment (Fig. 1). We conducted analytical studies to test the rice production hypothesis.[6]

ANALYTICAL STUDIES TO TEST HYPOTHESIS

1. Agricultural chemicals

Correlation between environmental pollution index (EPI) and SMRs of BTC

The amounts of agricultural chemical products distributed to 47 prefectures were obtained from "Noyaku Yoran".[7] Total amounts of each agricultural chemical product were divided by total prefectural area and the quotient obtained was designated as the environmental pollution index (expressed in kg/km^2 or l/km^2) of the chemical product. The EPIs of each chemical product from 1962 to 1975 were calculated yearly for individual prefectures and then related to the SMR of BTC for the corresponding prefecture in 1985 by using Pearson's correlation coefficient analysis after the logarithmic transformation of the EPIs and SMRs.[8]

Table 1 shows the correlation coefficients between the EPIs and SMRs. Of about 500 chemicals listed in "Noyaku Yoran", the EPIs of MCPA-E (4-chloro-2-methylphenoxyacetic acid ethylester), MCPB-E (4-chloro-2-methylphenoxybutyric acid ethylester), CNP (2, 4, 6-trichlorophenyl 4′-nitrophenyl ether), NIP (2, 4-dichlorophenyl 4′-nitrophenyl ether) and PCNB (pentachloronitrobenzene) were frequently associated with the SMRs. Interestingly, the chemicals which were found statistically significant were almost the same every year. They are characterized as the phenoxy compounds (MCPA-E, MCPB-E), diphenylethers (CNP, NIP) and phenol (PCNB). In addition, it should also be emphasized that they are all related to chemicals which have been reportedly contaminated with dioxins.

The present analysis does not allow us to determine how the use of these chemicals is causally related to the occurrence of BTC, since the present analysis is an ecological correlation type, which does not indicate the relationship at an individual level. In order to elucidate the causal relationship, the fate of these chemicals and dioxins, and their effects on humans should be investigated.

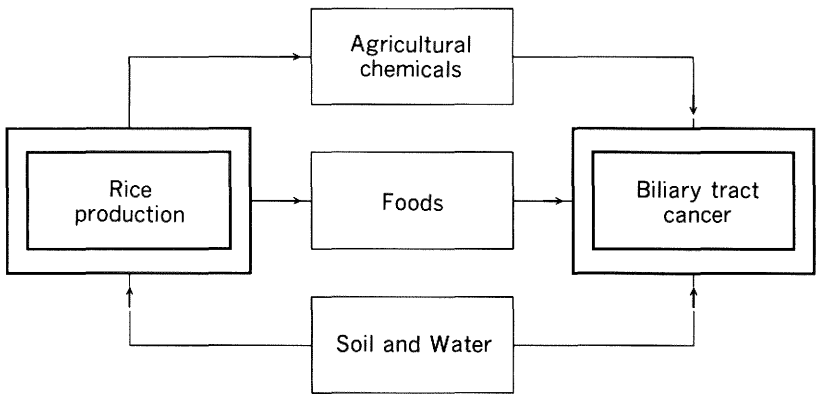

Fig. 1. Rice production hypothesis for the etiology of biliary tract cancer in Japan

Table 1. Correlation coefficients[a] between environmental pollution index (EPI)[b] of agricultural chemicals[c] (1962-1975) and SMRs[d] for billiary tract cancer (1985).

Year	MCPA-E		MCPB-E		CNP		NIP		PCNB	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1962	0.130	0.285	−0.310*	−0.311*	—	—	—	—	−0.090	−0.304*
1963	0.203	0.375*	0.392**	0.442**	—	—	0.058	0.066	0.251	−0.092
1964	0.361*	0.177	0.334*	0.482***	—	—	0.077	0.174	0.099	0.198
1965	0.363*	0.281	0.250	0.450**	—	—	0.091	0.253	−0.156	0.018
1966	0.327*	0.333*	0.237	0.133	—	—	0.172	0.285	0.237	0.073
1967	0.321*	0.278	0.116	0.076	0.180	0.157	0.214	0.393*	0.163	0.266
1968	0.386**	0.300*	0.160	0.062	0.146	0.067	0.246	0.389**	0.210	0.367*
1969	0.385**	0.192	−0.223	−0.136	0.202	0.246	0.296*	0.471***	0.264	0.214
1970	0.315*	0.395**	−0.110	−0.145	0.300*	0.368*	0.270	0.345*	0.279	0.353*
1971	0.502***	0.328*	0.168	−0.090	0.450**	0.401**	0.216	0.302*	0.290	0.258
1972	0.492***	0.323*	0.034	−0.086	0.495***	0.427**	0.267	0.325*	0.466**	0.532***
1973	0.520***	0.346*	0.384**	0.225	0.528***	0.456**	0.233	0.222	0.437**	0.431**
1974	0.553***	0.366*	0.359*	0.304*	0.529***	0.444**	0.162	0.350*	0.399**	0.457**
1975	0.593***	0.315*	0.393**	0.148	0.559***	0.492***	0.043	0.290	0.362*	0.367*

[a] Pearson's correlation coefficients after the logarithmic transformation of EPIs and SMRs. Significant levels: $*p<0.05$, $**p<0.01$, $***p<0.001$

[b] EPI was calculated by dividing the total amount of component annually distributed to each prefecture by its area, and expressed as l/km^2 or kg/km^2.

[c] MCPA-E: 4-chloro-2-methylphenoxyacetic acid ethylester, MCPB-E: 4-chloro-2-methylphenoxybutyric acid ethylester, CNP: 2, 4, 6-trichlorophenyl 4′-nitrophenyl ether, NIP: 2, 4-dichlorophenyl 4′-nitrophenyl ether, PCNB: pentachloronitrobenzene.

[d] Standard population is the total population of Japan in 1985.

Phenoxy compounds

As part of the investigation into the biological characteristics of MCPA-E and MCPB-E, mutagenicity and co-mutagenicity, hepatotoxicity and carcinogenicity were examined.

(1) Mutagenicity and co-mutagenicity

The Ames' test was employed to determine the genetic effects of these compounds. The mutagenicity test was done by using Salmonella typhimurium strain TA100 and TA98 with or without S9 mix. The co-mutagenicity, which is referred to as the enhancement of the mutagenic action of 2-aminoanthracene (2 μg/plate) by MCPA-E and MCPB-E, was tested using TA100 and TA98 only with S9 mix. The dose ranges of MCPA-E and MCPB-E were 2.89-185 and 4.06-260 μg/plate in the system with S9 mix, 0.0452-2.89 and 0.0317-2.03 μg/plate in the system without S9 mix, respectively. The results were as follows: they enhanced the mutagenic action of 2-aminoanthracene, although they were not mutagenic.[9]

(2) Hepatotoxicy

Effects of MCPA-E and other phenoxyacid compounds (MCPA, 2,4-D and Clofibrate) on hepatic xenobiotic metabolizing enzymes were studied in male rats. These compounds were administered orally 200 mg/kg/day to the rats for 2 weeks. MCPA-E and other compounds increased the hepatic level of cytochrome P-450. MCPA in particular increased the levels of not only P-450, but also Cytochrome B_5 and NADPH-cytochrome C reductase activities.[10] We also found that MCPA and its related compounds induced hepatic peroxisomal enzymes in rats.[11] Inomata[12] extended his research to investigate the effects of MCPA on the bile composition of golden hamsters. When MCPA was adiministered orally to the animals at doses of 200, 500 and 1,000 mg/kg/day for 2 weeks, the levels of cholesterol and the proportions of secondary bile acids, such as deoxycholic acid and lithocholic acid, increased in the bile. It is suggested that the increased levels of cholesterol in bile may lead to the formation of gallstone (GS) and that the secon-

dary bile acids have promotional actions in carcinogenesis.

(3) Carcinogenicity

Takagi[13] examined the carcinogenicity of MCPA in male mice. MCPA soaked in pellets was given to them for 18 months at the levels of 40, 200, 1,000 and 5,000 ppm (about 3.9, 19.0, 95.6 and 566.4 mg/kg/day, respectively). The mice were then placed on a control diet for 12 months. Autopsy was performed immediately after death or sacrifice, when were moribund or the experiment was completed.

Histopathological examination revealed that leukemia was observed in mice treated with 40 ppm MCPA. No effects on the gallbladder, except for mucosal papillary proliferation, were noted.

(4) Cancer epidemiology

Timonen and Palva[14] reported a case of acute myelomonocytic leukemia after exposure to MCPA. On the other hand, there is insufficient epidemiological evidence for a causal relationship between the use of phenoxy compounds and the occurrence of neoplasms.[15]

Up to the present, our review of the literature reveals that the occurrence of BTC in relation to exposure to MCPA has not been reported. To our knowledge, no report has been made regarding MCPB.

Diphenylethers

NIP was registered in Japan in 1963 and has been used since. However, it was deregistered in 1982 because of reports of carcinogenic potential in the U.S.A. CNP, which is produced by chlorination of NIP, was approved for use in Japan in 1965. More than 93% of the total products of CNP have been used in Japanese paddy fields.

First, the levels of CNP and its derivative, CNP-amino in drinking water were analyzed in Niigata and Joetsu Cities (high and low SMR areas, respectively).

The reason why CNP-amino was taken as a subject of analysis was that mutagenicity was reported in Ames' test after hepatic activation.[16] Secondly, a review of the literature was made as to the carcinogenicity of diphenylethers.

(1) CNP and CNP-amino concentrations in the drinking water

The levels of CNP and CNP-amino concentrations in drinking and river water collected from Niigata and Joetsu Cities were examined. Sampling was undertaken bi-weekly from the beginning of April, 1992, when the application of CNP to paddy fields started. Sampling was continued until the CNP and CNP-amino concentrations in water returned to the background level of April. Two liters of water were extracted using SEP-Pak C18 cartridge, and CNP and CNP-amino in the extract were measured with the ECD-gaschromatography (Shimadzu 9A). The quantity limits of CNP and CNP-amino were 0.25 and 10 ng/l (ppt), respectively. Finally, confirmation of the presence of these chemicals was made by GC/MS (Hewlett-Packard GC 5890 Series II).

As shown in Table 2, the maximum levels of CNP concentrations were 871.16 and 554.24 ng/l in river and drinking water, respectively, in the first week of May in Niigata City. The concentration of CNP in

Table 2. CNP concentrations[a] in river and drinking water collected from Niigata and Joetsu Cities[b].

Month	Week	Niigata City		Joetsu City	
		Shinano River	Drinking Water	Sekikawa River	Drinking Water
April	1st	1.16	U. Q.[c]	1.38	6.10
	3rd	0.77	1.21	7.61	5.04
May	1st	871.16	554.24	182.62	2.09
	3rd	15.04	57.47	21.16	3.17
June	1st	14.63	20.51	6.73	5.15
	3rd	4.65	8.20	8.79	6.02
July	1st	3.04	5.59	3.50	3.83
	3rd	2.84	2.68	0.82	5.34
	5th	0.28	3.00	46.03	8.63

[a] The concentrations were expressed as ng/l (ppt).
[b] Samples were collected bi-weekly from April to July, 1992.
[c] Under quantity limit; CNP concentrations were less than 0.25 ng/l.

drinking water was well correlated with that in river water. The concentration of CNP returned to the background level in the 5th week of July. In Joetsu City, the maximum concentration in river water was 182.62 ng/l in the 1st week of May and decreased rapidly towards the background level in the 3rd week of July, but increased again to 46.03 ng/l in the 5th week of July. As to the concentrations of CNP in drinking water in Joetsu City, these fluctuated between the levels of 2.09 and 8.63 ng/l. The peak corresponding with the maximum one in the river water was not observed.

In the case of CNP-amino concentrations in drinking water, a clear-cut difference was evident between the two cities (Table 3). In the drinking water in Niigata City, the maximum concentration was 384.21 ng/l in the 3rd week of May, with a gradual decrease observed towards the 5th week of July. In contrast, the concentrations in Joetsu City were always under the quantity limit.

The reason why there are differences in CNP and CNP-amino concentrations in drinking water between Niigata and Joetsu Cities is that the sources of drinking water are from the Shinano or Agano river in the former, and from reservoirs located in the mountains or underground water in the latter case.

The presence of several cities with insignificant SMRs even in the midst of the Niigata plains prompted us to examine the CNP and CNP-amino concentrations in tap water collected from these cities. The sample collection was made on May 16 or 17, 1993, and a gas-chromatographic analysis was made. For comparative purposes, concentrations of these chemicals in tap water collected on the same day in Niigata City were also analyzed, where SMRs were high.

As shown in Table 4, the concentrations of CNP were 76.47, 3.34, 6.09, 50.68 and 8.76 ng/l in cities of Niigata, Murakami, Gosen and the two areas of Kamo, respectively. Those of CNP-amino were 325.45, 17.03, 23.01, 159.75 and 22.26 ng/l in corresponding cities. It is apparent that concentrations of CNP and CNP-amino are higher in tap water where the source is the Shinano river (Niigata City and a part of Kamo City) than where the source is from groundwater (Murakami and Gosen Cities), or the Kamo river originating from the mountains (a part of Kamo City).

(2) Review of carcinogenicty of diphenylethers

Quest et al.[17] evaluated the carcinogenic potential of diphenylether herbicides (Fig. 2). CNP was not listed in their review, however, probably because CNP was originally produced and primarily consumed in Japan.

All the chemicals listed in their review produced liver tumors in mice. In particular, Lactofen and Nitrofen produced liver and pancreas tumors, respectively, in rats. BTC was not reported in mice. It should be noted that rats lack a gallbladder.

Since all of the diphenylether herbicides have carcinogenic potential in mice and some in rats, carcinogenic elucidation of CNP should be required. To the present, there is only one report[18] with regards to the chronic toxicity and carcinogenicity studies of CNP. According to this report, hyperplastic nodules of the liver were present in mice and rats treated with CNP for 2 years. Neither histopathological description of hyperplastic nodules nor the final conclusion as to whether or not CNP has carcinogenic potential was

Table 3. CNP-amino concentrations[a] in river and drinking water collected from Niigata and Joetsu Cities[b].

Month	Week	Niigata City		Joetsu City	
		Shinano River	Drinking Water	Sekikawa River	Drinking Water
April	1st	U. Q.[c]	U. Q.	U. Q.	U. Q.
	3rd	25.19	U. Q.	10.50	U. Q.
May	1st	156.98	270.80	243.99	U. Q.
	3rd	127.25	384.21	34.02	U. Q.
June	1st	221.80	130.41	37.12	U. Q.
	3rd	101.70	123.98	45.36	U. Q.
July	1st	43.24	79.79	29.68	U. Q.
	3rd	53.42	35.21	22.95	U. Q.
	5th	41.61	28.29	35.64	U. Q.

[a] The concentrations were expressed as ng/l (ppt).
[b] Samples were collected bi-weekly from April to July, 1992.
[c] Under quantity limit; CNP-amino concentrations were less than 10.0 ng/l.

Table 4. CNP and CNP-amino concentrations[a)] in tap water collected[b)] from cities with and without statistically significant SMRs[c)] of gallbladder cancer on the Niigata plains.

City	SMR Male	SMR Female	Location of sampling (Source)	CNP	CNP-amino
Niigata	190.08*	153.72*	Asahi-machi (Shinano river)	76.47	325.45
Murakami	162.51	92.45	Tabata-machi (Groundwater)	3.34	17.03
Gosen	128.63	128.63	Iseno-kawa (Groundwater)	6.09	23.01
Kamo	116.84	119.70	Miyakoga-oka (Shinano river[d)])	50.68	159.75
			Goban-cho (Kamo river)	8.76	22.26

a) Concentrations are expressed as ng/l (ppt); Quantity limits are less than 0.25 and 10.0 ng/l in CNP and CNP-amino, respectively.
b) Samples were collected on May 16-17, 1993.
c) Standardized mortality ratio of the gallbladder cancer (1981-1990); Population of Japan in 1985 was used as a standard; * $p<0.001$
d) Water supply from the Shinano river started in 1973.

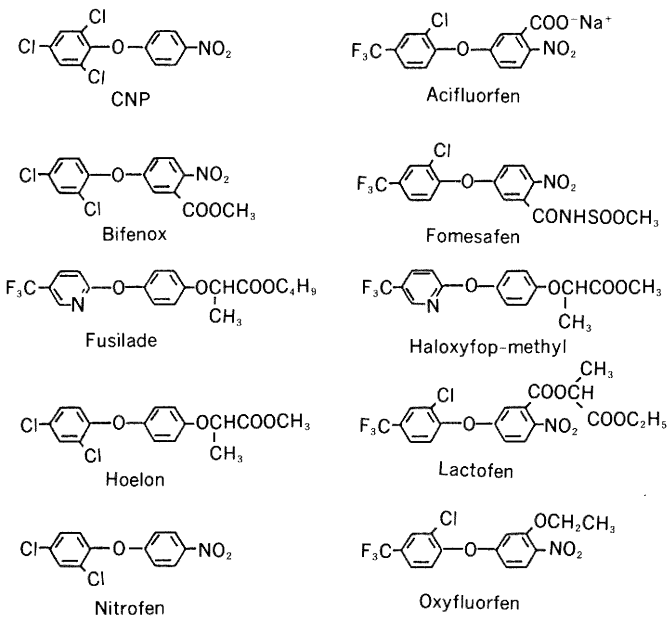

Fig. 2. Chemical structure of CNP and related diphenyl ether type herbicides [modified from Quest, J. A. et al. (1989)].

made in this report.

In addition to the carcinogenic evaluation of CNP, another diphenylether herbicide, chlomethoxynil (2, 4-dichlorophenyl 3′-methoxy-4′-nitrophenyl ether) should also be examined. As the use of chlomethoxynil was started in 1975, it was not picked up in our ecological correlation analysis.[8)]

PCNB

Since the ecological correlation analysis revealed the statistical association between the use of PCNB and SMRs of BTC, PCNB was taken into consideration at the initial stage of investigation. In the course of

our investigation, however, it was found that the SMRs of Nagano, Ibaraki and Gunma Prefectures, where a larger amount of PCNB were used than Niigata, were not as high as those in Niigata. Secondly, the possible route of human exposure was via the contamination of vegetables in the case of PCNB, since it is used as a fungicide for them. If so, the statistical association between the frequent consumption of contaminated vegetables and BTC should have been apparent in the case-control study.[19] Based on these considerations, the role of PCNB on the occurrence of BTC was withdrawn as a candidate for an etiological agent.

Dioxins

As impurities of MCPA-E, MCPB-E, CNP, NIP and PCNB, which were found to be associated with the occurrence of BTC in an ecological correlation analysis, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans are suspected. Among these impurities, the carinogenicity of 2, 3, 7, 8-TCDD has been a matter of concern. These compounds, however, are less likely to contain 2, 3, 7, 8-TCDD, since appropriate precursors like 2, 4, 5-T are not present. As an impurity of CNP, 1, 3, 6, 8-TCDD should be reserved for future carcinogenic evaluation.

2. Characteristics of food consumption in rice producing areas

Ecological correlation analysis

By using principal co-ordinate analyses on 47 prefectures, Endoh et al.[20] revealed that the consumptions of sodium chloride and dried noodles were positively and those of beef, eggs, ham and bread were negatively correlated with SMRs of BTC. It may be worthwhile deducing that the people living in prefectures with high death rates consume less westernized food, their diet characterized as low in fat and protein.

If such a less-westernized diet should actually be responsible, it is likely that people who favor low-fat foods retain bile juice for a while in the gallbladder. This interpretation, however, may be clearly contradicted by the recent increase in death rates in spite of the change in dietary habits towards high fat consumption in Japan.

Nutritional analysis of meals

We collected 20 samples of identical meals ("Kagezen" in Japanese) from Shiunji Town (located on the Niigata plains) and Maki Village (located outside the Niigata plains), and analyzed the contents of fat, protein and carbohydrate in these. The proportions of fat, protein and carbohydrate were 19.8%, 14.7% and 65.5% in Shiunji Town and 17.8%, 12.2% and 70.0% in Maki Village, respectively. No difference in the proportion of each nutrient was evident between them, whereas the death rates of BTC varied considerably.

3. Geological Characteristics

Trace elements in river sediments in Niigata Prefecture

We investigated the distribution of four trace elements, i.e. selenium (Se), molybdenum (Mo), arsenic (As), and tin (Sn) in samples of river sediment from 19 points near drinking water sources of major cities and towns in Niigata Prefecture. The reasons for the selection of the four trace elements are: 1) it is reported that mortality from cancer is inversely correlated with various environmental materials[21]; 2) a similar association between Mo and cancer has been suggested; 3) the interaction between Se and As is well known; and 4) As and Sn are thought to belong to the cacinogenic metal group.

The samples were digested with the use of perchloric and hydrofluoric acids and the concentrations of metals were then determined by means of atomic absorption spectrometry (AAS), and in the cases of As, Se, and Sn, AAS with a hydride yielding device. The mean concentrations of Se, Mo, As, and Sn were 0.44 ± 0.187 (mean$\pm$S.D.), 3.82 ± 1.031, 1.23 ± 0.781, and 4.12 ± 2.861 ppm, respectively. Dividing Niigata Prefecture into two areas, the Niigata plains and the other parts of Niigata Prefecture showed that there was no remarkable difference in the concentrations of three trace elements, Se, Mo, and As, between the two areas. Se was 0.45 ppm in Niigata plains and 0.42 in the other parts of Niigata; Mo was 3.85 and 3.72 ppm; As was 3.90 and 4.73 ppm. The sole difference was found in Sn concentration, this being 1.49 ppm on the Niigata plains and 0.76 ppm in the other parts of Niigata Prefecture. The analyses of association between BTC, GBC, and BDC and the four trace elements did not show any inverse correlation.

Selenium (Se) contents in human bile

Chen et al.[22] analyzed Se contents in human gallbladder bille. Thirty seven subjects were studied: 22 patients with gallstone(GS) in Niigata Prefecture and 15 patients (13 with GS and 2 with gallbladder polypus)

in Kochi Prefecture. The reason why these two prefectures were selected for sampling is that the SMRs of BTC (1983–1987) vary considerably between them: 127.0 in males and 138.6 in females in Niigata, and 85.7 and 69.3, respectively, in Kochi.

Five ml of bile was withdrawn with a syringe from the gallbladder during the operation and stored at −20℃ until analysis. For analysis by gas chromatography with ECD, 0.2 ml of sample was used. The mean Se concentrations in bile were 269±39.0 (mean±S.D.) ng/ml in Niigata and 285±84.4 ng/ml in Kochi without any significant difference.

4. Other Factors

Table 5 shows the factors which have been reported before. Some of the factors, such as GS, cholecystitis (including typhoid fever), anomalous arrangement of pancreaticobiliary duct and clonorchiasis sinensis are clearly associated with BTC, but others were not consistent in terms of the association with BTC.

Gallstone and cholecystitis

Kato et al.[19] presented interesting findings in their case-control study. The past histories of these diseases were listed as risk factors both in GBC and BDC. Odds ratios of GS were infinite, as the control group had no GS. In case of cholecystitis, they were about 34 and 13 in GBC and BDC, respectively. We have to say, however, that their results are inconsistent with the prevalence of GS diagnosed by ultrasonic screening of residents in Niigata prefecture. Even in the Niigata plains where the mortality from BTC is high, the prevalence of GS among residents over 40 years old is about 2.3%, which hardly differs from the average in Japan. In regard to the role of cholecystitis including typhoid fever, there had been no episode of epidemics in these areas.

In summary, a big gap in results between the case-control and ecological correlation analyses exists. A multifactorial causation hypothesis, which will be

Table 5. Summary of epidemiological studies on cancer of the biliary tract.

Factors[a]	Comments
1. Genetic factors	• Orientals (American Indians and Japanese); HLA-DR4 in patients with gallbladder cancer
2. Sex (Female > Male)	• Pregnancy; Gallstone formation in females
3. Ageing	• Definite risk factor
4. Gallstone	• Definite risk factor (Supported by case-control studies in Japan. The prevalence of gallstone disease is not high in Niigata Prefecture.)
5. Anomalous arrangement of pancreaticobiliary ducts	• Mainly associated with bile duct cancer
6. Infection (Cholecystitis & Typhoid fever)	• Definite risk factor (Supported by case-control studies in Japan. Their prevalence rates are not high in Niigata Prefecture.)
7. Parasite infection (Chinese liver fluke)	• Mainly associated with bile duct cancer
8. Ulcerative colitis	• Mainly associated with bile duct cancer
9. Other diseases (Cystic fibrosis, Ataxia telangiectasia, Gastrectomy)	• Only case reports
10. Simple food	• Foods which contain less fat and protein
11. Drugs (Clofibrate, Isoniazid, Methyl-DOPA, Chenodeoxycholic acid, Oral contraceptive pill)	• Mostly case reports; Clofibrate (Intervention study, n.s.); Pill (Case-control study, n.s.)
12. Occupation	• Workers exposed to asbestos, trichloroethylene and other industrial chemicals
13. Agricultural chemicals	• Suggested by an ecological correlation analysis. Phenoxy- and diphenylether- herbicides are constantly associated with SMRs of biliary tract cancer. Carcinogenicity of diphenylethers has been investigated from various aspects in our laboratory.

[a] Do not always indicate a causal association

mentioned later, may explain this discrepancy.

Clonorchiasis sinensis

The Niigata plains used to be an endemic area of clonorchiasis. In recent times, however, neither any episode of the disease, nor the presence of flukes at autopsy has been reported. It seems unlikely that clonorchiasis is the only explanation of the geographical variation of the death from BTC in Niigata.

Anomalous arrangement of the pancreaticobiliary duct (AAPBD)

The distribution of AAPBD on the Niigata plains (high SMR area) has not been investigated in relation to BTC. Nevertheless, it seems less likely that it is responsible for the clustering of BTC, since AAPBD is congenitally determined and there is no particular reason to believe that the residents on the Niigata plains are different from those who live in other parts of Niigata.

ANALYSIS OF RESULTS AND FORMULATION OF NEW HYPOTHESIS

1. Validity of rice production hypothesis

As a link between rice production and the occurrence of BTC, agricultural chemicals, dietary pattern, geological characteristics of soil and drinking water and other factors have been evaluated. The investigation into the role of each factor gave some suggestive findings. None of the factors, however, seems to act as a single cause in the etiology of BTC. These factors may instead interact with others. We shall therefore try to summarize the results from the point of view of both host and environmental aspects. Thus, those who have a past history of GB or cholecystitis are high risk groups. When they are exposed to geographically specific environmental factors, such as agricultural chemicals in rice producing areas, they may develop BTC. The present inference is a sort of multifactorial causation hypothesis.[23]

2. Additional study to characterize host factors

HLA analysis of BTC

Before the introduction to the new hypothesis, HLA analysis was performed to examine whether or not a genetic factor was involved in the development of BTC.

Yamamoto et al.[24] made a report on HLA antigens in GBC. Thirty one patients with GBC and 32 healthy controls were typed using antisera against 12 HLA-A, 31 HLA-B, 7 HLA-C, and 13 HLA-DR antigens. The DR4 frequency of 61.3% (19/31) in GBC was significantly different from that of 28.1% (9/32) in the control. The relative risk and the etiologic fraction were 4.0 and 0.46, respectively.

We extended our HLA analysis of BTC and obtained the final results as shown in Table 6. We examined HLA-A, B, C and DR antigens in GBC and BDC, but none of the phenotype frequencies of HLA-A, B and C in these diseases was significantly different from that in the controls. Only in the case of HLA-DR antigens was a significant difference detected. The frequency of DR4 in GBC was 61.5% (24/39), which was significantly different from the 30.0% (12/40) in the control group. In the case of BDC, however, the figure of 28.1% (9/32) was not different from that of the control.

3. Multifactorial causation hypothesis

First of all, two distinct findings should be pointed out: one is that the clustering of BTC in Niigata is due to GBC[4] (female GBC in particular),[5] and the other is that HLA-DR4 is associated only with GBC.[24] Based on these, it may be worthwhile considering the pathogenesis of GBC and BDC separate-

Table 6. Phenotype frequencies of HLA-DR antigens in patients with gallbladder and extrahepatic bile duct cancers.

Antigens[a]	Control (n=40)[d]	GBC[b] (n=39)	BDC[c] (n=32)
DR1	3 (7.5)	2 (5.1)	5(15.6)
DR2	19(47.5)	12(30.8)	13(40.6)
DR4	12(30.0)	24(61.5)[e]	9(28.1)
DRw6	7(17.5)	8(20.5)	4(12.5)
DRw8	11(27.5)	8(20.5)	8(25.0)
DRw9	13(32.5)	8(20.5)	9(28.1)
DRw11(5)	3(7.5)	3(7.7)	3(9.4)
DRw12(5)	9(22.5)	7(17.9)	4(12.5)
DRw13(w6)	0	1(2.6)	4(12.5)
DRw14(w6)	0	1(2.6)	1(3.1)

[a] Of 13 antigens typed, 10 positive ones in any of the 3 groups are listed.
[b] Gallbladder cancer.
[c] Extrahepatic bile duct cancer.
[d] No. of cases examined.
[e] p=0.0046 (Fisher's exact test of probability).
p=0.046 (Corrected p value).

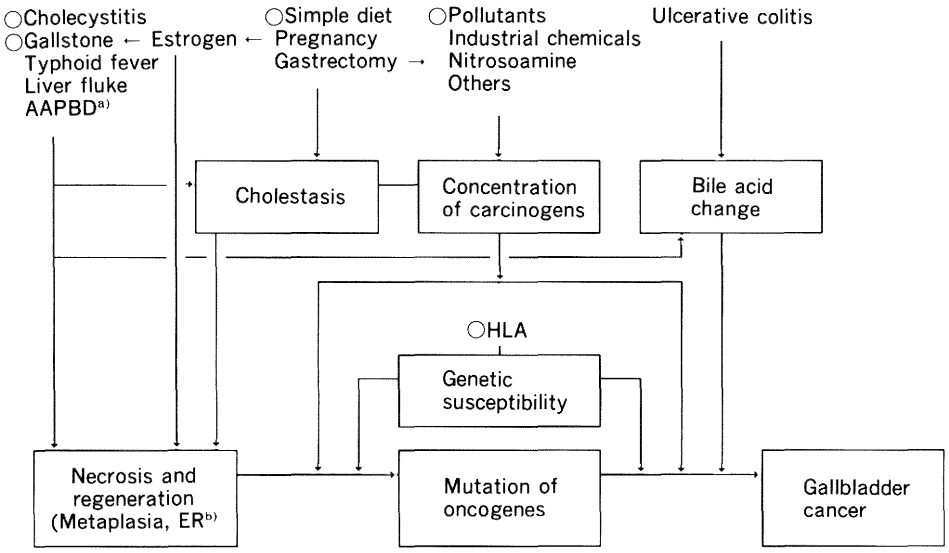

Fig. 3. Multifactorial etiology of gallbladder cancer in Niigata, Japan.
a) Anomalous arrangement of the pancreaticobiliary duct, b) Estrogen recepter, c) A combination of host factors (e.g. genetic susceptibility, past histories of cholecystitis or gallstones, preference for low-fat diet) and factors in the environment (e.g. agricultural chemicals) may determine the occurrence of gallbladder cancer in Niigata, Japan.

ly. In this paper the pathogenesis of GBC has focused upon the reasons mentioned above.

In addition to the factors disclosed under the assumption of the rice production hypothesis, various others have also been taken into consideration to formulate a new one. Fig. 3 illustrates an interaction of factors which have been reported as a pathogenesis of GBC. A plausible explanation for GBC in Niigata is as follows: GBC may occur when those who have genetic susceptibility (e.g. HLA-DR4) and past histories of gallbladder disease (e.g. GS or cholecystitis) are exposed to geographically specific factors, such as agricultural chemicals. The waterborne route via the contaminated Shinano or Agano rivers is highly suspect.

Based on the results obtained by an analysis of ecological correlation between the use of agricultural chemicals and SMRs of BTC and gaschromatographic analysis of drinking water, diphenylether herbicides such as CNP and its impurities should be placed under suspicion for the occurrence of GBC after 1965, when CNP was introduced to the environment.

FURTHER TEST OF NEW HYPOTHESIS

The results of analytical studies generated a new multifactorial causation hypothesis. Prior to testing this hypothesis, it was necessary to identify an environmental factor or factors which prevailed in the Niigata plains. Among several factors examined, it should be pointed out that CNP and CNP-amino concentrations in drinking water in Niigata City (located on the Niigata plains) are higher than those in Joetsu City (located outside the Niigata plains). These findings may be explained by the different sources of drinking water; from the Shinano and Agano rivers for Niigata City, and from reservoirs in mountains and underground water for Joetsu City. This conjecture may be supported by findings that the sources of drinking water were not from the Shinano or Agano rivers but from the underground water or rivers originating from mountain areas in the low SMR areas such as Murakami, Gosen and Kamo Cities in spite of their location in the midst of the Niigata plains. An analytical study should, therefore, be directed to focus on the route of exposure via drinking water collected from the Shinano and Agano rivers.

The first question to be asked is what contaminants are ultimately responsible for the occurrence of BTC (GBC in particular) on the Niigata plains. Since the diphenylether herbicides and their impurities are the most suspected chemicals, the regional differences in concentrations of these chemicals should be examined in relation to the clustering of GBC (test of

the strength of association).

The second consideration is whether or not the diphenylether herbicides and their impurities have carcinogenic petential. Up to the present, no animal studies indicating whether these chemicals induce GBC in particular have been available. We therefore designed an animal model on the basis of the multifactorial causation hypothesis where a suspected chemical is given to golden hamsters whose gallbladders are surgically operated to have "experimental GS". The aim of this protocol is to examine whether or not the combination of nonspecific injury of gallbladder mucosa and chemical treatment per os induces GBC (Test of the biological plausibility). If we were to find positive results, an appropriate subsequent study would be to stop using these chemicals in paddy fields and observe changes in the incidence or death rate of GBC between the Niigata plains and the other parts of Niigata Prefecture (test of the temporally association). It would also be important to examine changes in other malignant neoplasms in order to point out the specific relationship between the use of diphenylether herbicides and GBC (test of the specific association).

The third possibility is to wait for the supplementary studies supporting our epidemiological analysis (test of the consistency of association).

Answers to these may further generate new ideas which could modify or replace the current hypothesis and pave the way for the clarification of the etiology of BTC (GBC in particular).

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