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Epidemiologic Approaches to Assessing Human Cancer Risk from Consuming Aquatic Food Resources from Chemically Contaminated Water

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Epidemiologic approaches to assessing human cancer risk from consuming fish from contaminated waters must confront the problems of long latency and rarity of the end point (cancer). The latency problem makes determination of diet history more difficult, while the low frequency of cancer as an end point reduces the statistical power of the study. These factors are discussed in relation to the study designs most commonly employed in epidemiology. It is suggested that the use of biomarkers for persistent chemicals may be useful to mitigate the difficulty of determining exposure, while the use of more prevalent and timely end points, such as carcinogen-DNA adducts or oncogene proteins, may make the latency and rarity problems more tractable.

Introduction

Cancer is the second leading cause of death in the U.S., claiming more than 1000 lives a day. Cancer is among a small set of dread diseases that claim special public attention because of deep-seated societal values, anxieties, and attitudes (*1*). The appearance of cancer among feral fish populations is alarming not only because it may be an indication of a seriously compromised aquatic habitat but also because of real or imagined consequences for the human population in the same food chain. In this paper, we discuss the use of epidemiologic techniques to study the effect on human cancer risk of consuming fish or shellfish from such aquatic environments.

Epidemiologic Approach

Our knowledge of harmful effects of environmental pollutants comes from three sources: clinical case descriptions, toxicological experiments, and epidemiological studies. For our purposes, the distinguishing characteristic of a toxicological study is the fact that it is an experiment, i.e., the investigator manipulates the independent variable, exposure, and observes the effect on some physiologic or pathophysiologic measure under controlled conditions. Environmental epidemiology, on the other hand, is an observational, not an experimental, science. The environmental epidemiologist finds a natural experiment occurring in the laboratory of the real world, observes its outcome, and then arranges the observations in a manner that produces the most information.

Toxicology and epidemiology are naturally complementary in the information they produce. As an experimental science, with observations made under controlled conditions, toxicological results usually have high internal validity, i.e., when properly designed they provide good and relatively unambiguous answers to the precisely framed questions of the experiment. Unfortunately, they may also be quite low in external validity, i.e., generalizability or extension to situations differing from the exact conditions of the experiment. The problems of extrapolating from rodents to humans or from high dose to low dose are both problems of poor or uncertain external validity.

Epidemiological experiments, by contrast, often have low internal validity. Because we are constrained to observe unplanned events in a setting that shares some but not all of the characteristics of a controlled experiment, we are often left with multiple, alternative explanations for the outcome of our unintended experiment. The results, however, by their natures are highly generalizable, as they pertain to humans living under natural conditions.

The very nature of the epidemiologic enterprise imposes constraints on the kinds of problems that can be effectively and efficiently addressed by this method. It is our task here to discuss epidemiologic approaches to the question before this conference in the light of these constraints and to suggest some possible strategies to overcome them.

Epidemiologic Study Design

Epidemiologic study design is the process of finding and systematically observing a natural experiment. There are three

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principal varieties of study design in common use, along with various hybrids of the main types (2).

The most familiar design, because of its similarity to the laboratory experiment, is the cohort or follow-up study. In this method, two groups of individuals differing in exposure are observed for a period of time and then compared with respect to their disease experience. If the two groups differed only in terms of exposure then the distinction between an observational study and a laboratory experiment would be subtle. Unfortunately, in most situations the two groups differ in many other ways as well, and these disparities must be minimized in the study design and in the analysis.

The second principal design is the case-control study. Here a group of individuals with a particular disease (the cases) are compared to a group of individuals without the disease (the controls) to see if their prior exposures differ. For this type of study to be valid the disease process must not influence the information on exposure. In the past, such designs were called retrospective studies, although now case-control or case-referent study is the preferred terminology. In both cohort and case-control studies, it is the exposure prior to disease that is considered, consistent with a cardinal rule of causality that cause precedes effect.

In the third type of study design, the cross-sectional study, disease and exposure status are determined at a single and concurrent point in time. This is appropriate when the exposure is some trait or characteristic that can plausibly be held to precede the development of the disease (e.g., blood type). In many instances, however, when comparing the prevalence of symptoms in an exposed group to that in a suitable unexposed group, one may be uncertain if a difference in symptom prevalence preceded the exposure. Nonetheless, compared to cohort or case-control studies, cross-sectional studies are less costly and easier to carry out and when properly designed and interpreted can yield very useful information.

A variant of the cross-sectional study is the ecological or geographic correlation study. Here the disease experience of populations in geographic regions that differ in some important exposure characteristic are compared. A difficulty with such studies is that there are usually many other differences between the populations besides the particular characteristic that prompted the comparison. An example would be a comparison of cancer rates between seaport populations and nonseaport populations based on the assumption that people in seaport areas consume more locally caught seafood. Seaport cities are usually industrial cities, however, and the circumstances that contaminate a harbor, river, or lake might also expose the population through the drinking water, the air, or in the workplace. Moreover, the industrial workforce, with its characteristic demographic makeup and lifestyle (diet and smoking, for example) may have no comparable counterpart in nonseaport populations. Hence, both the population and critical elements of the environment might differ in addition to any difference due to consumption of locally caught seafood.

Regardless of design, a knowledge of the statistical power of the study is important to interpretation of results. Statistical power is the ability to show with confidence that a true effect, as opposed to one that occurred by chance, is present. Several factors determine the statistical power of an epidemiologic study, including the magnitude of the effect under study, the prevalence of exposure in the population, the frequency with which the disease under study occurs, and the number of subjects. The relative importance of these factors will vary according to the type of study. If the disease of interest is rare, often a case-control design is used, but if the exposure is also uncommon then the case-control study will have little power. Similarly, if exposed and unexposed populations can be assembled for a cohort study but few cases of the disease of interest occur during the observation period, then this approach too will have little power. Low power means that unless the association of the disease with exposure is strong, it is unlikely to be statistically demonstrable.

For example, assume that exposure to fish contaminated with a potent cause of liver cancer could be measured with perfect accuracy. If 10% of the population of Boston ate enough fish to have their risk of liver cancer increased by 50%, for a study to have at least a 90% chance of detecting this increase we would have to wait more than 10 years for enough cases of liver cancer to accrue before we could undertake a case-control study.

Cancer Epidemiology and Consumption of Fish from Contaminated Water

For long latency diseases, like cancer, where the development of a solid tumor is usually in excess of 15 to 20 years after exposure to a carcinogen, information must be available over a significantly long period of time. Thus, for a cohort study we must either wait several decades for the disease to develop or assemble a decades-old cohort with ascertained historical exposure and trace them to the present. Similarly, for a case-control design, we must be able to ascertain exposure in our cases and controls at a time decades before diagnosis of the disease.

How well can we determine for each individual how much of the residue-laden fish he or she consumed 20 years ago? There are two problems here. The first is to determine how much fish an individual consumed 20 years prior, the second to determine if the individual consumed contaminated fish.

Food intake is recalled with less than perfect accuracy. Most of us have difficulty remembering what we ate in the very recent past, much less 20 years ago. However, we can determine broad patterns of food consumption (dietary habits), and dietary recall methods have been successfully employed to detect rather subtle food and nutrient effects. We will therefore rephrase the question as: How well is fish intake measured relative to other foods?

In four studies, the reproducibility or validity of questionnaires regarding fish intake have been examined. Thompson et al. (3) resurveyed subjects after 15 years and found that, compared to other foods, fish intake was recalled fairly accurately. In this study the frequency of fish consumption was divided into eight categories; about 45% of subjects chose the same category at baseline and when they recalled their intake 15 years later. Jensen et al. (4) also resurveyed subjects after approximately the same length of time. This group found that fish consumption was recalled more accurately than most other foods (the Spearman correlation coefficient between the amount of fish consumed at each of the two determinations was 0.41). Jain et al. (5) examined the correlation between diet records and diet histories covering the same recent period of time. The intake of fish was reported about as accurately as the intake of other foods

Spearman coefficient for fish, 0.62, for shellfish, 0.72). These studies show that fish and shellfish are remembered as well or better than other foods and that recent diet is more clearly recalled than diet many years earlier. By contrast, Pietinen et al. (*6*) found that in Finland fish intake was reported less accurately than most other foods. Fish intake for this population was more than 40 g/day, which is unusually high. The fact that fish tends to be less frequently consumed in the United States may contribute to the fact that it is better remembered.

To date, these methods have not established firm associations between fish consumption and cancer. We reviewed all epidemiologic studies ($n = 46$) in which fish or shellfish intake was examined in relation to cancer of the lung, large bowel, pancreas, breast, upper aerodigestive tract, stomach, endometrium, ovary, and prostate (*7–53*). We were unable to find similar studies of sarcoma or hepatoma. Data regarding salted or smoked fish were excluded. For cancers of the bowel, esophagus, stomach, breasts and pancreas, the evidence reveals no relation between fish intake and increased or decreased risk. For cancers of the lung, upper aerodigestive tract (excluding esophagus), endometrium, ovary, and prostate, results were inconsistent and there was an insufficient number of studies on which to base a generalization. Thus, at this time the epidemiologic evidence does not support an association, either detrimental or beneficial, between fish intake and cancer risk.

The second important question in any study of fish consumption from contaminated waters concerns the extent to which the source of the recalled diet can be determined. This depends on the likelihood that the individual knows and can recall the source (e.g., a recreational or commercial fisherman who habitually consumed a portion of his catch) or the possibility that fish or seafood consumed in a particular geographic location will have a source that can be determined.

To illustrate the first possibility, one might take advantage of the fact that the Commonwealth of Massachusetts issues a special license for recreational lobstermen. Some 12,000 of these licenses have been issued since 1972. One could imagine assembling a cohort of such individuals who are likely to know the source of at least some of the shellfish they have eaten. Either the lobstermen as a whole could be considered exposed and a follow-up done with cancer registry or mortality data, or an attempt could be made to contact them, take a dietary history, and subsequently follow them for an appropriate period of observation. The latter option, although more expensive, would permit an exposure categorization to be made internal to the cohort, allowing comparisons solely within the group of lobstermen. Recently, Jones et al. drew a 1% random sample ($n = 1600$) of 1984 fishing license holders in Wisconsin (*54*). Fishing and fish consumption were determined by questionnaire, and blood levels of DDE (a metabolite of DDT) and polychlorinated biphenyls (PCBs) were determined. Although response rate was only 50%, the feasibility of the method was clearly demonstrated.

The precise source of fish and shellfish in a particular georaphic locality is even more difficult to determine. Even if it could be demonstrated that most locally consumed fish were also locally caught, there is likely to be considerable spatial variation in the contamination of local supplies (*55*). The result of these uncertainties will produce exposure misclassification with a consequent reduction in the sensitivity or power of the study.

In summary, it is possible to measure fish consumption in the past by recall methods, but as expected, the more distant the recall required, the less reliable the data. The bias produced by this loss in accuracy due to misclassification reduces the statistical power of the study, making it less likely to show that a true association is statistically significant; however, it will not produce a spurious association.

Strategy of Biological Markers: A Measure of Internal Dose

It is important to remember the environmental context of the problem: exposure of a human population to substances through the food chain. Some substances that cause tumors in fish or shellfish may not be passed up the food chain to human populations because they are completely metabolized or sequestered at lower trophic levels. For example, it is likely that most polyaromatic hydrocarbons (PAHs) do not find their way to humans via this route (*56*). On the other hand, poorly degraded and metabolized substances such as PCBs or heavy metals will do so. It is plausible that substances that persist at the lower trophic levels may also do so in humans, i.e., what leaves a residue in fish will also leave a residue in people. This is a generalization that may have some exceptions but is sound as a basis for strategy.

This fact suggests that we obtain data on carcinogenic residues in humans as a surrogate for data on consumption levels of contaminated fish. Because we must apply this method to a relatively large population, we need an accessible and easily obtainable tissue. Fat and muscle biopsies are likely to be unacceptable to many people but may be useful for validation or case-control studies where the number of subjects is not very large. Blood, saliva, and urine are possible sources for analysis, although most contaminants that will find their way up the food chain will not be easily eliminated by kidneys or salivary glands. Moreover, obtaining urine specimens is usually more difficult than obtaining blood samples. Breast milk from lactating mothers also is an appropriate source of material for analysis. In addition, metals may be detected in hair and nails.

Persistent organics, such as PCBs, are best measured by fat biopsies. In the absence of this method, blood serum levels or milk from lactating mothers may be used. Studies of the relationship between ingestion of PCB-contaminated fish and PCB levels in blood serum and milk have shown that these levels do indeed correlate with exposure (*57–59*). In one of the studies, a variety of factors that might affect PCB blood levels were examined, and it was found that age, sex, and amount of fish consumption (in that order) were the most important (*60*). PCBs can also be passed from mother to infant through the placenta or breast milk (*59,61,62*). It seems to be well established that higher exposure to PCBs means a higher blood level of PCBs. The studies that evaluate levels after exposure ceases are not consistent, but it appears that the higher chlorinated congeners are eliminated much more slowly, over months to many years.

Among the toxic metals that have been found in fish and shellfish are cadmium, chromium, copper, lead, and mercury (*63–65*). Blood levels are widely used and accepted as a measure of exposure to lead (*66*), and blood levels for Cr^{3+} are elevated after occupational exposure (*67*). Methyl mercury exposure

correlates highly with levels in blood (*68*), but for copper and cadmium it is unclear if blood levels reflect exposure until frankly toxic intakes are reached (*69,70*).

Methyl mercury exposure also correlates well with mercury levels in hair (*71*), suggesting that levels in toenails might also be useful, especially as hair may be affected by various treatments, such as permanent waving. Toenails are especially convenient to collect and store. Moreover, in the U.S., toenail are usually sheltered from the environment by shoes, making trace element analysis less subject to interference from environmental contamination. Toenail cadmium levels have been used as an accurate measure of above normal exposure (M. Maclure, unpublished data), but the relation between chromium and copper exposures and nail levels is much less well defined (*68*).

With the exception of lead, these trace elements can be measured nondestructively and simultaneously by neutron activation analysis using very limited amounts of sample (*72*). While the metals themselves may not be carcinogenic, they may serve as useful markers for exposure to organic carcinogens, especially if a fingerprint of trace metals can be determined that is characteristic of exposure to contaminated fish or seafood. In general, depending on which digit is sampled, nail levels reflect body levels in the preceding 3 to 12 months. This relationship may be especially useful in a case-control design since blood analyses would measure current body levels that might have been affected by the disease process itself (*73*).

Whatever the indicator of internal exposure, a study that correlates consumption of carcinogen-contaminated fish and shellfish with an appropriate marker is an important part of the overall strategy. It is obvious that selecting a marker will require cooperation between laboratory scientists and epidemiologists.

Markers of Biological and Preclinical Effect

Markers of internal exposure may alleviate some of the problems that result from exposure misclassification, but the difficulties caused by seeking end points or exposures of low frequency remain. To address the problem of low frequency end points such as cancer, either large sample sizes are required, or another consequence of exposure, more common than cancer, must be used. If, in addition, another appropriate end point appeared earlier than cancer we could use dietary recall methods for a shorter period and hence with more confidence.

In recent years, several techniques to measure higher prevalence end points associated with cancer have been suggested (*74*). It is believed that the earliest steps in malignant transformation by chemical agents involve covalent binding of the substance to cellular DNA. Fish sampled from PAH-contaminated areas of the Buffalo and Detroit Rivers have elevated liver cancer rates and have demonstrably higher levels of aromatic carcinogen-DNA adducts (*75*). Detection of cellular carcinogen-DNA adducts in humans by highly specific antibodies has also been accomplished for a few agents and holds some promise for use as an epidemiologic end point (*76*). Detection of adducts in urine resulting from DNA repair processes has also been successful, although those who excrete the adducts may arguably be at lower risk because of their obvious DNA repair competency (*77*). Chemicals that alter DNA may also alter proteins. Adducts on readily accessible proteins such as hemoglobin or albumin have been used as surrogates for DNA adduction. The much larger quantity of protein available increases the minimum detectable level of adduction (*78*).

Other end points closer to the clinical event may also prove useful. Sister-chromatid exchanges, chromosome breaks, or increases in the point mutation rate have all been suggested, although they are not agent specific, nor is their relationship with the cancer end point clear. Recently, the use of oncogene proteins as preclinical response indicators has been advocated (*74*). This technique assumes that inappropriately timed or regulated expression of particular cellular genes or the products of a mutated gene are the key events in the malignant process. In some cases, both the genes and the products associated with human tumors have been identified. Thus, monoclonal antibodies directed against an abnormal gene product might be used to screen cohorts for those individuals at highest risk of developing cancer. The validation of this method remains to be accomplished, however. Indeed, before any of these techniques can be used in epidemiologic studies, a great deal of work must be done to document the sensitivity and specificity of the methods as indicators of either exposure or biological effect (*79*).

Conclusion

It is not yet possible to give a confident answer to the general question of whether cancer risks are increased by consuming fish and shellfish from chemically contaminated waters, although in particular instances we may be able to make some reasonable judgments. As this conference illustrates, determining cancer risk will require persistent effort from many different directions. Because in this setting epidemiologic studies have limited power, the extent to which they can play a role appears minimal for the immediate future. An attempt to quantify exposure to chemical carcinogens with biologic markers in populations consuming relatively large quantities of contaminated fish or shellfish may be a reasonable starting point.

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