OCCUPATIONAL CANCER

Modifying Factors In Experimental Lung Cancer

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Lung cancer is the leading cause of cancer deaths among American men. Cigarette smoking is presumed to be the most significant etiologic factor in this cancer toll. In addition, several occupations have been associated with an excess risk of lung cancer. Examples that are well known to all of you include uranium mining, asbestos production and application, production of bis-chloromethyl ether, coke oven operation, production and polymerization of vinyl chloride, smelting and refining of chrome, nickel, arsenic, etc. It has been shown that some of these occupational exposures when combined with cigarette smoking yield a greater cancer risk than a similar type of exposure in the absence of smoking, suggesting additivity or synergy of their effects. Yet not everyone who is subjected to both cigarette smoke and the occupational exposure develops lung cancer. The variability of the carcinogenic effects, even in individual situations at a very high risk, suggests a biologic variability within the exposed population. This variability may be predicated on several physiologic or biochemical properties of the individual that influences either the actual biological dose or the response to a given exposure. Such factors as extent and duration of smoke inhalation, numbers and types of cigarettes and the distribution of smoking throughout the day may be important.

With regard to occupational exposures: the use of protective equipment, the specific job function, the source or location of the material being used, and even the physical design and ventilation of the worksite may influence risk. Individual characteristics, such as rate of function of pulmonary clearance mechanisms, extent of cellular loss from a given injury, and the rate of repair of self-proliferation, capacity for cellular DNA repair, and nutritional and immunologic status may further influence individual risk. And since genetic diseases that predispose to cancer development are known, it is conceivable that the variability of these capacities may be genetically determined. Previous disease or intercurrent illnesses can also increase the risk of respiratory tract cancer.

Because individuality and variability are hallmarks of the human race, it is difficult to isolate the specific effects of the plausible variables to determine if and how they contribute to the risk. For these reasons we turn to experimental models of human cancer—especially, human lung cancer--to gain further understanding. There are several experimental models for respiratory tract carcinogenesis. Each has its strengths and weaknesses. I've chosen to begin this discussion with one that I am most familiar with.

One of the early lessons in experimental chemical carcinogenesis studies was that tumors take a long time to develop, just like human cancers. Toxicity of the carcinogens and intercurrent infections of the animals hampered early efforts. Thus development of what was to become a very widely used model for respiratory carcinogenesis was marked by two significant advances; first, the Syrian golden

hamster. These animals were found to be resistant to the epizootic chronic respiratory infections of conventional rodents. They don't develop spontaneous lung tumors and they are relatively tolerant to instillations of carcinogens into the respiratory tract. Secondly, the carcinogen in our studies—usually benzopyrene—are attached to a particulate material such as ferric oxide dust; the mixture is then instilled directly into the respiratory tract. The result of the new methodology was that tumors developed in the respiratory tract. Furthermore, the animal tumors resemble—in morphology and location—the majority of tumors found in the human respiratory tract. Then too, the tumors appear to develop as a result of a histogenesis process through a sequence of lesions resembling those associated with the tumors of the respiratory tract in humans. And finally, tumor response is reasonably quantitative and reproducible, and the relationship between dose, response and latency is reminiscent of the disease in humans.

In practice, benzopyrene is physically adsorbed onto iron oxide particles suspended in saline. The suspension is injected by syringe into the trachea of the experimental animals. Particles of iron oxide/benzopyrene become distributed throughout the lung. A single injection of the mixture has a toxic effect on tracheo-bronchial epithelium. There is a selective loss of ciliated cells from the epithelium initially; an early response involves basal cell hyperplasia with an increase in the number of basal cells as well as simplification of the overlying epithelium followed by non-keratinizing squamous metaplasias. With continuing injections, a large area of tissue becomes squamous metaplastic with distinct keratinization at its surface. For the most part, these are reversible lesions. Continuing treatment leads to damage of the respiratory epithelium and simplification of the epithelium with toxic changes in the nuclei. Basal cell hyperplasia and focal areas seem to grow extensively. Basal expansion progresses from a relatively well-ordered and reasonably differentiated stage to extensive lesions with many fewer superficial differentiated cells. In time, the entire thickness of the epithelium is composed of basaloid cells that represent an in situ carcinoma. Malignant cells in an early, small evasive lesion show rather limited suffusion through the underlying stroma. As time and treatment progress, there is more extensive infiltration throughout the underlying stroma. Even where the malignant tumor is localized, the entire underlying soft tissue becomes suffused with malignant cells.

One question of technical importance is related to the role of dust particles in the experiment. To address this issue, studies were made in which benzopyrene was mixed with dust by alternate methods. The results showed an increased risk of tumor induction when there was an actual physical association (by grinding) between the carcinogen and the particles, as opposed to simply mixing benzopyrene with the particles or to benzopyrene alone. Adding additional dust didn't seem to affect the outcome. Other particles (such as carbon and nickel) were also compared to ferric oxide. There was a much higher tumor incidence with carbon than with ferric oxide. Perhaps the surface adsorptive characteristics of the particles are a factor. It has been hypothesized that the particles slow the clearance from the respiratory tract, thus increasing the carcinogen dose acting on the respiratory tract. Experimental studies that have evaluated the clearance of carcinogen from the respiratory tract tend to support this role for the particles. It is conceivable, therefore, that conditions that are apt to reduce the clearance of materials out of the lung contribute to the effect of the dose of carcinogen. Cancer risks might then be lowered by altering contributory factors as well as acting on the carcinogen itself.

Hence, several anesthetic agents were evaluated to determine if there might be a simpler way to anesthetize animals in these studies. Ether has generally been avoided

because it is a pulmonary irritant. Sodium barbitol is typically used as a parenteral solution; it yields deep anesthesia and reduces respiratory rate without irritation to the respiratory tract. Methoxyflurane is an inhalation anesthetic which also does not cause pulmonary irritation and is effective as a moderate level anesthesic, causing less reduction of the ventilatory rate and a more rapid rate of recovery.

Groups of hamsters treated with benzopyrene/ferric oxide while anesthesized by these various agents were compared; for the most part, hamsters treated with either barbitol or ether gave a higher incidence of tumors than those given methoxyflurane. Since both ether and barbitol gave a deeper level of anesthesia than methoxyflurane, it was thought that they might cause a reduced clearance rate for the carcinogen mixture. Again, if the carcinogen mixture is removed more slowly from the respiratory tract, then the integral of dose with time of exposure is greater for ether and barbitol than for methoxyflurane; this is the equivalent of giving a larger dose of carcinogen. Again, there is the suggestion that the state of pulmonary function as it affects clearance of carcinogens influences the carcinogenic risk in the experimental animals.

Another factor that has been shown to influence lung cancer incidence in humans is one's concurrent exposure to more than one carcinogen. Experimental studies have examined interactions between carcinogenic treatments under the controlled conditions of an animal study. In each case benzopyrene was adsorbed on ferric oxide particles in one of the treatments. Other co-carcinogens included diethylnitrosamine, which is a metabolically activated carcinogen with great organ specificity for the respiratory tract; methylnitrosourea, which need not be metabolized to be active and is not specific for the lung; and polonium-210, an alpha-particle emitter. Hamsters that received small doses of benzopyrene adsorbed on ferric oxide displayed a very low incidence of malignant tumors. When a benzopyrene/ferric oxide treatment was followed by subcutaneous injections of diethylnitrosamine, malignant tumors occurred earlier and in significantly greater numbers. Note that diethylnitrosamine per se did not yield malignancies.

In the second study with benzopyrene/ferric oxide followed by methylnitrosourea, there was a greater tumor response than that produced by either agent alone; the individual agents actually gave rather low tumor incidences. When the results of these two treatments were reevaluated, it was observed that the tumor incidences exceeded the expected interaction of the two carcinogens acting alone. This suggests a non-additive effect if it occurred in a linear dose-response relationship. However, this type of study does not distinguish an additive response from a synergistic effect on a non-linear dose response curve.

The third experiment employed benzopyrene/ferric oxide with polonium-210. Here there was a slightly increased tumor incidence which appeared to be a simple additive effect. If we consider all these interactions, they suggest that:

> Carcinogen interactions can be studied by experimental model systems.
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> Interactions are not simple; they may vary according to the specific agents and with such factors as the sequence of the agents and their timing.
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> The experimental model offers an opportunity to study these effects as they may bear upon human exposures to multiple agents. Reasonable methods may then be developed for reducing lung cancer deaths by eliminating or modifying the most critical points of the exposure equation without necessarily having to avoid exposures to all possible carcinogens.

Other factors may also modify tumor incidences. For example, if vitamin A-deficient animals have increased carcinogen binding to DNA within the respiratory tract, they also get more lung tumors. Human epidemiologic studies also have shown that men who eat a diet relatively deficient in vitamin A have an increased risk of lung cancer. Thus, vitamin A deficiency appears to be a predisposing condition for tumor development in the respiratory tract. On the other hand, animals supplemented with high doses of vitamin A after benzopyrene treatment developed fewer tumors than those treated with benzopyrene alone. Let me warn, however, that both natural vitamin A and the vitamin A tablets sold over-the-counter are highly toxic if taken in large doses, and may even be fatal. Studies are underway to develop vitamin A analogues having beneficial anti-cancer effects and lessened toxicity.

Related studies of the chemical prevention of carcinogenesis have evaluated other antioxidants, such as butylated hydroxytoluene, butylated hydroxyinositol and disulfuram (or antabuse). Experimental studies of peripheral lung adenocarcinomas and adenomas with mice is not the exact equivalent of the majority of lung cancers in man. It's been found that chronic treatment with these antioxidants in the diet reduces lung tumor incidence. The lung tumor response can also be modified in the hamster model by influencing the immune system of the animal. We have treated hamsters with benzopyrene/ferric oxide and immediately thereafter immunized them with isolated cell walls of the BCG bacillus. Inoculations yielded a substantial, non-specific immune stimulation in the treated animals. Moreover, the immunized hamsters developed fewer lung tumors than those not immunized.

Thus, modification of activity of the cellular immune system may also influence tumor responses. We have learned that the rate of induction of lung cancer is predicated upon a complex interaction between the exposure to various carcinogenic factors and host factors. The somewhat disparate list of factors that are able to modify experimental models of carcinogenesis is indicative of the wide range of individual factors that influence the tumor development.

When the problem is looked at holistically, it's not surprising that a single, simple theme doesn't emerge. The phenomenologic studies do contribute to our understanding of the carcinogenesis process. Three distinguishable elements in the process can be identified: exposure, susceptibility and modulation. Factors such as attachment of carcinogen to particles, dose of exposure and multiple exposures influence the quantity of carcinogenic insult that is encountered by the individual, and by reduction of exposures one can hope to reduce the toll of cancers. Dietary supplements of antioxidants may reduce one's susceptibility and reduce the initiated cancer cell quantity. Measurable tumor response in animals and perhaps in man may be modulated. Many factors, such as continued carcinogen exposure, repetitive injury or tumor promotion, may increase the number of tumors that actively grow and appear clinically, or they may even reduce the latency period for tumor initiation. One can not uncritically apply these experimental results; however, they do help to identify more profitable epidemiologic studies, suggest changes in environmental or industrial control, guide careful development of chemo-preventive procedures and, hopefully, help us to bring lung cancer under control.

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