Methodologic issues, theoretical considerations, and design criteria for experimental animal and cell culture experiments

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ABSTRACT This article provides background information that is important when evaluating the relevance to humans of particular animal or in vitro experiments designed to assess the relations between fatty acids and cancer. Considerations in designing carcinogenesis studies to assess the relation between dietary fatty acids and human cancer include selection of the animal model and design of the experimental diets. Animal carcinogenesis models are generally best for evaluating the early phases of cancer development: the initiation and promotion of cancer. Transplantation protocols have been developed for evaluating the effect of diet on the growth and metastasis of partially or fully transformed cells. The variables that are important in such models are the origin and biology of the cell line, the animal host used for the implantation, the site of transplantation, whether the primary tumor is excised after a period of time to allow for metastasis, and when the diets are fed relative to the different phases of tumor growth and metastasis. Studies in cultured cells have been particularly useful for assessing the mechanisms by which fatty acids affect cancer. Considerations in designing studies with cultured cells include selection of the cell line, cell culture conditions, selection of biological endpoints that are relevant to human cancer, and in vivo confirmation of the mechanisms observed in vitro. Design considerations for each of these experimental approaches are discussed and the contributions of each approach are summarized. Am J Clin Nutr 1997;66(suppl):1506S-12S.

KEY WORDS Fatty acids, cancer, experimental studies, methods, rodents, cell culture, animal experimental studies

INTRODUCTION

Animal models of cancer have been useful in assessing the role of the type and amount of dietary fat, particularly dietary fatty acids, in cancer development and progression. Studies conducted in animals, with cultured cells, or with cells transplanted into animals have been used to identify mechanisms for the effects of dietary fatty acids on cancer. Understanding mechanisms of dietary modulation of cancer is necessary to establish whether dietary fatty acids are a causative factor in human cancer. Animal investigations and in vitro studies can be designed to explore specific aspects of the influence of fatty acids on cancer. These studies must be carefully interpreted in relation to the experimental design when results are related to the situation in humans.

This article provides background information important for evaluating the relevance to humans of particular animal or in vitro experiments on fatty acids and cancer; it includes an overview of design considerations in animal and in vitro studies and a discussion of the relevance of such studies to the relation between individual fatty acids and cancer. This article also reviews the relevance of fatty acids to carcinogenesis in skin and pancreas because these models will not be covered in other articles in this supplement.

CARCINOGENESIS STUDIES

Consideration of the animal model

Selection of a relevant animal model is central to the design of a carcinogenesis study. The model should, if possible, use an agent for the induction of cancer that is suspected as a cause of human cancer. Because our knowledge of the factors related to several forms of human cancer is limited, choosing a relevant agent is sometimes a problem. Recent data have implicated heterocyclic amines in human cancer, making the development of models of breast cancer with heterocyclic amines potentially relevant (1). Aflatoxin induction of liver cancer has long been viewed as a relevant model for human liver cancer (2). However, if a relevant induction model is not available, as in the case of pancreatic cancer, it is preferable to use an agent that will induce cancer with only a short exposure. In that way, the period of exposure to the carcinogen can be separated from the period of exposure to the experimental diet. Models with potent carcinogens, however, may produce results different from those in humans because of the associated toxicity of the agent in the animal model.

Pancreatic cancer in humans occurs primarily in ducts (3), but many of the animal models of pancreatic cancer have acinar cell tumors (4). One exception is the hamster pancreatic cancer model induced by bis(2-oxopropyl)nitrosamine by both multiple- and single-treatment protocols. In studies of the influence

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METHODS FOR STUDIES OF FATTY ACIDS AND CANCER

of amount of dietary fat on pancreatic cancer, Birt et al (5) followed a single treatment of bis(2-oxopropyl)nitrosamine with feeding of either control (4.2% by weight) or high-fat (20.5% by weight) amounts of corn oil. Feeding the high-fat amount resulted in a two- to fourfold increase in the incidence and number of pancreatic carcinomas (5). Further studies with this model showed that the enhancement of cancer in the hamsters fed the high-fat diet was not due to excess energy consumption by that group (6). Studies with this model also showed that corn oil, which is high in unsaturated fatty acids, was not more effective in the enhancement of ductular pancreatic cancer in hamsters than was beef tallow, which is rich in saturated fatty acids (7). Studies by Roebuck et al (8), in which azaserine was used to induce acinar cell tumors, supported a role of diets high in unsaturated fatty acids in the promotion of acinar pancreatic cancer. These studies further showed that linoleic acid (18:2n–6) was particularly effective in the promotion of acidophilic acinar foci, with the yield of tumors increasing above a threshold of 4–8% dietary 18:2n–6 (9).

Short-term treatment protocols can also be used to study the effect of diet on initiation, promotion, and progression. Such models, however, although useful in sorting out mechanisms, do not actually reflect the situation in humans. Humans are notable for being long lived, allowing for exposure to a wide variety of agents that may influence cancer risk over the life span. People are exposed to agents that may be important in cancer causation over the entire period of cancer development rather than at discrete times. Furthermore, the stages of human cancer are defined in terms of the acquisition of genetic aberrations in the developing lesion (10). Tumor models for colon cancer generally require multiple doses of carcinogen (11), possibly because of the need for sequential genetic damage in the induction of colon cancer.

High-dose carcinogen treatment over a short time is commonly used for experimental carcinogenesis to shorten tumor latency. Such a protocol was compared with a low-dose protocol in mammary carcinogenesis studies by Jacobson et al (12). Their results showed a longer latency period with low-dose treatment. However, animals fed a high-fat diet developed more mammary tumors over a shorter time than did animals fed a low-fat diet, irrespective of carcinogen dose. Autopsies of the animals fed a high-fat diet showed that those animals treated with low doses of carcinogen had a range of breast lesions that was more typical of what is seen in human breast cancer patients, whereas those treated with higher carcinogen doses had mainly adenocarcinomas.

The chemically induced two-stage model of skin carcinogenesis that requires a single topical treatment of 7,12-dimethylbenz[a]anthracene followed by repeated treatment with the promoter 12-O-tetradecanoylphorbol-13-acetate has been used to determine the effect of dietary fat on initiation and promotion events. However, this model does not represent human skin cancer, which is primarily induced by exposure to ultraviolet light from the sun (13). Contradictory results were obtained from different laboratories in studies of the importance of dietary fat and 18:2n-6 intake in modifying chemically induced skin carcinogenesis (14). Factors such as time between the initiation and promotion phases and when during promotion a diet rich in 18:2n-6 is fed may determine whether an enhancement (15) or an inhibition (16) is observed. Studies of squamous cell skin cancer induced by ultraviolet light in mice showed that the consumption of diets rich in 18:2n-6 resulted in a higher incidence than did the consumption of diets rich in menhaden oil (17).

Animal models should also have pathologic, immunologic, genetic, and pharmacologic features similar to those of the disease in humans. For example, the induction of breast fibroadenomas is not a good model for human breast cancer because human breast cancers are adenocarcinomas. Use of a model that has gene mutations also found in human cancer is believed to be more relevant than use of models involving different mutations. Intermediate markers of carcinogenesis have been identified for several carcinogenesis models (eg, aberrant crypts for colon cancer and hyperplastic alveolar nodules for breast cancer). Such intermediate markers can be useful for assessing the efficacy of intervention strategies. Intermediate markers in experimental models should reflect lesions observed in the progression of the disease in humans.

When the carcinogenic agent is known to induce human cancer, dietary studies on initiation may be particularly relevant to people. A series of studies aimed at dietary prevention of liver cancer began with the observation that oltipraz, a drug that induces hepatic glutathione sulfotransferase activity and reduces hepatic aflatoxin-DNA adducts, was effective in preventing liver carcinogenesis by aflatoxin in rodents (18). The relevance of these observations for people with chronic exposure to aflatoxin-contaminated foods is currently being tested in China in studies designed to use oltipraz supplementation as a strategy for reducing liver cancer rates.

Studies on dietary fat and carcinogenesis have primarily assessed postinitiation events because of the paucity of information on the agents responsible for human cancer and because most studies suggest that this phase of carcinogenesis is sensitive to dietary fatty acids. The postinitiation phase may be more affected by dietary fatty acids because of longer exposure. Studies on metastasis have primarily used transplantation models, as discussed below.

In a few studies the exposure to diet was investigated not only at different times relative to carcinogenic exposure, but also during in utero development and throughout life. This research is important but extremely difficult to design so that it relates to human cancer. It is essential to know whether the pregnant animal model and humans handle carcinogenic agents and dietary constituents similarly. Differences between humans and the species being used with respect to dietary requirements for development must be considered. Species differences in growth factor secretion and responsiveness likely contribute to differences in sensitivity to carcinogenesis.

Attention is currently focused on how hereditary factors affect the risk of cancer. Animal models for human genetic susceptibility are being developed as information is acquired on the genetic lesions in human cancer. The animal models that have been used for cancer research clearly represent genetic susceptibilities. Specific animal species and strains are generally more susceptible to a given carcinogen. Unfortunately, the particular genetic traits that are important are usually not known. For example, estrogen-induced pituitary tumors in rats are observed only in certain rat strains, including Fischer 344 rats; other rat strains, such as Holtzman and Sprague-Dawley, have been found to be estrogen-resistant (19). Multiple genes were found to be responsible for the susceptibility (19), and efforts are being made to uncover the genetic basis of this strain
difference. Animal models of human genetic susceptibility are being developed by using approaches such as stable transfection, gene knockout, and humanizing (expressing human genes in the animal tissue) animal models. The aim is to have models that represent the complexity of the genetic factors in human cancer. The availability of such models will facilitate animal investigations that examine the relation of specific fatty acids to cancer development and improve interpretation of the results from animal studies.

Considerations in the design of experimental diets

A basic consideration is whether a semipurified or a natural-ingredient diet should be used to achieve the objective of a particular study. Natural-ingredient diets have the advantage of containing food ingredients consumed by humans. Such diets are particularly useful for assessing the interactions between food constituents and the relation of these interactions to cancer. The disadvantage of such diets is that it is impossible to control the complex interactions between food components. Changing only one or two components at a time in such diets is virtually impossible. However, natural-ingredient diets are particularly useful for unraveling important interrelations between dietary components and assessing the effect of food components on bioavailability.

Purified diets are usually used when studying the relation of specific fatty acids to cancer development. Purified diets reduce the number of components that can interact with the fatty acids of interest. However, complex interactions can occur with purified diets. For example, if dietary fat and fiber interactions are under investigation and dietary fiber amounts are to be increased, the investigator must ensure that the consumption of other nutrients is constant. As diets higher in fiber are consumed, some animal strains will reduce their consumption because of the bulk of the high-fiber diet. Thus, the intake of all other components is reduced. Another concern with high-fiber diets is the effect that such diets have on absorption of other dietary components such as minerals and fatty acids.

Particular attention to dietary interactions is required for proper formulation of high-fat diets. The foremost consideration is how the additional fat will be substituted into the diet. In the past many investigators substituted fat for carbohydrate by weight (20). This resulted in a high-fat diet that was also high in energy density. If the animals consumed the same energy as did those on the low-fat diet, they consumed considerably less of all other nutrient components. However, energy intake should be kept constant when comparing low- and high-fat diets in animals. Species such as rats appear to adjust their consumption of high-fat diets to consume the same amount of energy as do control animals over short-duration experiments (21). This is not true of all species. For example, hamsters freely fed a high-fat diet gained an average of 9% more weight than did freely fed control animals (22). Diet formulation as well as species may be factors in an animal’s ability to adjust its energy intake. Furthermore, cancer studies are generally of long duration, and small differences in energy intake may be important over the long term.

Energy intake is a major factor in the development of cancer, and studies of dietary fat must be controlled so that energy intake is the same in control and experimental groups. Body weight may not be a good indicator of dietary energy intake in some species. Although rats tend to adjust food consumption to compensate for differences in energy density when they are young and growing, they tend to lose that ability as they age, and become heavier when fed a high-fat diet (12).

Effects of dietary energy and fat are tightly interwoven because on a weight basis fat contributes about twice as much energy as do carbohydrate and protein. In addition, deposition of dietary fat in tissues requires less metabolic energy than does conversion of protein or carbohydrate to fat. Dietary fat contributes a significant proportion of the energy in most human diets. Thus, substituting a high intake of a lipid or fatty acid into an animal diet increases the energy intake of the animals as well as the fat intake. Because dietary energy is a potent modifier of cancer, with a small increase in energy intake generally resulting in a dramatic increase in cancer rate (23), it is essential when comparing low- and high-fat diets or different fatty acids that the diets are isoenergetic. With isoenergetic diets, the intake of the animals consuming the most energy (usually the high-fat group) is matched to the intake of the control group. This is done by caging the animals individually and pair-feeding the animals (individually or by group). Although this condition is necessary for proper dietary control, individual housing is an artificial condition for rodents and may influence results of the study.

Comparison of different fatty acids by substitution of one fatty acid for another avoids some of the problems inherent in substituting fatty acids for carbohydrate. The energy density and the relation between lipids and other components of the diet remain constant between diets. However, if the fatty acids have considerably different molecular weights, an equal molar substitution is not being made. Long-chain saturated fatty acids are less well absorbed than shorter-chain and unsaturated fatty acids, and very-short-chain fatty acids are absorbed directly into the blood whereas medium- and long-chain fatty acids are absorbed into the lymphatic circulation. These differences can have profound influences on the uptake and metabolism of fatty acids by tissues. Of course, some of these differences may be important to test in different experimental designs.

Another challenge is selection of the control fat amount and composition. If the objective of studying fatty acids is to compare diets that are adequate, then the investigator must ensure that the fatty acid requirement of the animal model is met in both the control and experimental diets. If the degree of fatty acid unsaturation is of interest, a diet containing fatty acids of the same chain length but different saturations will serve as a useful control. In most carcinogenesis studies, diets are fed over a long period of time, making the cost of purified fatty acids prohibitive; therefore, a control fat with a fatty acid composition that maintains optimal health but keeps cost down is usually selected. In many studies of dietary fat and carcinogenesis, control diets contain only one dietary lipid, such as corn oil, soy oil, or safflower oil. Although these diets provide a standardized control, people do not eat comparable diets. One approach has been to blend a number of fats in an attempt to mimic the overall fatty acid consumption of a human population group. The American blend (a mixture of dietary lipids formulated to replicate the average consumption of the US population) was available for experimental animal studies in the past. The drawback of using blended fats for a control is the difficulty maintaining uniformity in the blend between lots.

Fatty acids can bind minerals, calcium in particular, in the intestine, an important interaction between dietary fat and other
dietary components (24). This interaction is hypothesized to result in reduced mucosal toxicity of the fatty acid. Thus, results obtained with a high-fat diet and high amounts of calcium may be different from those obtained with a high-fat diet and lower amounts of calcium.

Unsaturated fatty acids are susceptible to oxidation before diet preparation, while in the diet, and after incorporation in animal tissues. Oxidation of fatty acids before the diet is fed is undesirable because of potential toxic properties of the oxidized fatty acids. Because of this problem of oxidation, antioxidants are added to fats rich in unsaturated fatty acids if they are not found naturally in these fats. Some plant oils are rich sources of fat-soluble antioxidants; for example, corn oil is rich in α-tocopherol and palm oil is rich in tocotrienols. However, with use of other dietary lipids, such as fish oil, antioxidants must be added to prevent oxidation of the many unsaturated lipids in the fat, including n−3 fatty acids.

Antioxidant concentrations must be constant in high-fat diets for the experimental results to be interpreted properly. However, the exact amount of supplementation that will prevent oxidation of the lipid and prevent oxidative stress in animal tissues is not known, and high amounts of antioxidants are usually recommended. Another difficulty is deciding whether the dietary antioxidant should be kept constant, which would provide extra antioxidant protection to the low-fat group, or whether the amount of antioxidant added to the diet should be added relative to the content of unsaturated fatty acid, which would provide extra antioxidant protection to the high-fat group.

Control diets and treatment conditions are generally selected to amplify differences between dietary groups. Although this is necessary to provide statistically significant differences, the results must be interpreted in light of this approach. For example, as pointed out by Ip (25), when a diet rich in 18:2n−6 is used as a control for mammary carcinogenesis, an observed inhibition in carcinogenesis relative to this control may be because the experimental diet provided less 18:2n−6 than the control diet did. Such results do not necessarily mean that the experimental lipid will protect against human breast cancer. A control dietary lipid should be selected carefully to be appropriate for the objectives of the experiment. There is no standard dietary control for experimental studies on lipids and carcinogenesis.

CANCER-PROGRESSION TRANSPLANTATION STUDIES

Models

Because metastases are lethal components of cancer progression, there is considerable interest in the effect of fatty acids on the growth and metastasis of cancers. In general, cancer progression—the final, irreversible stage of cancer—involves additional genomic instability in the developing cancer that increases malignancy and eventually results in metastasis (26, 27). One serious disadvantage of most animal models of cancer is that chemically induced tumors metastasize late in the process of cancer development. Conventional animal husbandry generally dictates that animals be killed when the first sign of carcinoma appears. For example, hamsters treated with bis(2-oxopropyl)nitrosamine will reduce their food consumption and begin to lose weight as a sign that they are experiencing the pain of the developing pancreatic carcinoma. Because pancreatic cancers develop internally, it is not clear how long the tumor has been present when signs of pain appear; however, when those signs do appear, only ~5% of the pancreatic tumors will have metastasized. Many hamsters with pancreatic cancer would eventually develop metastasis, but this is a late event and most hamsters are killed before metastasis occurs. Thus, animal carcinogenesis models are generally best for evaluating the early phases of cancer development—the initiation and promotion of cancer.

Protocols have been developed for evaluating the effect of diet on the growth and metastasis of partially or fully transformed cells. The variables that are important in such models are the origin and biology of the cell line, the animal host used for the implantation, the site of transplantation, whether the primary tumor is excised after a period of time to allow for metastasis, and when the diets are fed relative to the different phases of tumor growth and metastasis. Early transplantation studies were generally not held in high esteem because pieces of tumor or mixed-cell populations were put into animals and the time until the death of the animal was a common endpoint. More careful attention to issues involving cell line and animal host selection have made studies on tumor progression and metastasis more feasible and informative.

Selection of cell line

Human tumor cell lines are often used to improve the applicability of the research to people. However, the animal model must be such that the host immunologic system does not reject the foreign cells. Examples are the athymic nude and severe combined immunodeficiency disease (SCID) mouse models, which are animal models with an ineffective immunologic response. Normal immunologic surveillance is eliminated because of the defective rejection response in the animal model. Athymic nude and SCID mice retain natural killer cell activity, which may be important in dietary modulation (28). Selecting a tumor line from a syngeneic host allows the investigator to put the cell line into a host with a competent immunologic response but one that will not mount a massive rejection reaction.

Considerations in selection of cell lines for transplantation studies include whether the cells are competent for metastasis and can progress to metastasis when put into the host or whether they will form a large tumor that will kill the host before metastasis can occur. An ideal model would allow implanted cells to grow into a tumor that then would metastasize in a permissive environment. In studies designed to assess dietary augmentation of breast cancer therapy, use of a cell line responsive to common therapeutic agents for breast cancer could be particularly informative. An interesting model for studying early stages of breast cancer is under development with the MCF-10AneoT human breast cancer line implanted into nude mice. This cell line is derived from proliferative human breast disease and will develop into a tumor with histologic features of carcinoma in situ and invasive carcinoma with further transformation (29). The factors that cause this further transformation are under investigation.

Cell lines that can form metastases on injection into an appropriate host can be used as experimental metastasis models. Cells are injected intravenously and seed in the lung or
other appropriate sites. Metastases can be counted on the surface of the lung. In some cases, these cell lines can also be used in spontaneous metastasis protocols, where they are implanted into a receptive site, grow into a tumor, and are resected once the tumor grows to the size at which metastases are expected. One problem with spontaneous metastasis is that it is not known whether the diet influences the size at which the tumor will form a metastasis or the tissues where the metastatic cells are targeted to grow. A relation between primary tumor size and metastasis can be eliminated if there is no correlation between the weight of the primary tumor at necropsy and the occurrence or severity of metastasis. The spontaneous metastasis model has the advantage that the dietary treatment of human cancer patients can be mimicked. Experimental diets that are expected to enhance or inhibit cancer metastasis can be administered when clinical signs would be expected or after surgical removal as part of the therapy protocol. This area of nutrition and cancer has received less attention than cancer prevention research that focuses on earlier events.

Another transplantation model determines the effect of diet on tumor growth by using a cell line that will grow but not produce apparent metastases. Although this model is widely used, its applicability to human cancer prevention and therapy is unclear. In humans, once a tumor becomes apparent, it is removed, therapy is begun, or both are done in an attempt to save the patient.

Another consideration in designing animal models to mimic cancer progression to metastasis is deciding where the tumor cells will be placed in the animal model. It would appear logical to put the cells in the site of the primary tumor (autologous transplantation). This approach has been successful in several models (eg, for breast, prostate, and colon cancer). However, autologous transplantation may lead to rapid rejection of cells. Ofentimes the only site where the cells can form a tumor that will eventually metastasize is in a fat pad, under the kidney capsule, or intradermally, sites that protect the growing cells from lymphocyte surveillance.

**Dietary treatment during tumor progression**

An advantage of the diversity of models for studying progression is that diets can be fed at any point in the process. For experimental metastasis models diets can be prefed to animals to determine how diet affects adherence and growth of the cells at the metastasis sites. In spontaneous metastasis models animals can be fed experimental diets throughout the growth and metastasis of the tumors, only during growth, or after tumor excision. All of the considerations regarding diet formulation mentioned above in the section on carcinogenesis studies are applicable to these models also.

One of the primary disadvantages of the transplantation models of tumor growth and metastasis is that the tumors generally grow, metastasize, and kill the host rapidly. Thus, the dietary modifications have only a short time to alter the course of disease progression. This is a particularly important disadvantage for studies of dietary fat and cancer because the different amounts and types of dietary fat require time to modify the tissue fatty acid composition and presumably to change metabolism. Thus, differences in dietary fat that could modify cancer growth and metastasis may not be observed in animal investigations in which growth and metastasis occur in a matter of a few weeks. Furthermore, the rapid growth of the primary transplanted tumor and associated metastasis may not mimic the situation of generally slower-growing human breast tumors. However, for screening dietary patterns that may increase or decrease cancer growth and metastasis, short studies are more economically feasible. Furthermore, tumor growth can be extended somewhat by decreasing the size of the tumor cell inoculum.

**ASSESSING MECHANISMS FOR HOW FATTY ACIDS AFFECT CANCER: STUDIES IN CULTURED CELLS**

Much useful information has been acquired from cell culture studies on the potential mechanisms for the effects of dietary fatty acid on cancer. Cell culture systems have used primary cells from animals, immortalized cells from animals and humans, and genetically altered cells obtained from immortalized cells. Information on cell culture studies is particularly important for linking research in animals to research in humans because parallel studies can be conducted in animal and human systems. Furthermore, in vitro studies are needed to provide biological plausibility for an association between a dietary factor and a disease process.

**Selection of a cell line system**

Primary cultures are useful for following cellular responses to in vivo dietary exposure. The use of primary cultures avoids the genetic abnormalities that are required for cells to be kept in culture. However, a principal limitation of primary cultures is the potential for damage to the cells during the isolation process. Enzymatic hydrolysis, required to harvest primary cells from the tissue of interest, may damage many cells irreversibly; cells that survive may need time in culture to establish more normal processes.

One of the reasons for using primary cultures is to study the effect of dietary exposure in vivo on processes that can best be evaluated in vitro. For example, I have been interested in the effect of dietary fat intake on membrane lipid metabolism. To study phospholipid turnover, it is necessary to pulse-label lipid intermediates in the pathway of interest. We found that cells from mice fed different diets maintained differences in phosphatidyl inositol turnover for 44 h after removal from the mice (DF Birt, unpublished observations, 1992). Studies by Spector and Yorek (30) showed that phospholipids in fatty acid–enriched human skin fibroblasts maintained their enriched content for 4-10 d when cultured in a serum-containing medium.

Apart from the potential for cellular damage during the isolation process, another difficulty with primary cultures is establishing cultures of the cells of interest. For example, hamster duct epithelial cells must be microdissected from ducts of the pancreas and held in culture for a couple of weeks to allow the duct epithelial cells to grow out from the dissected ducts (31). In summary, although the use of primary cultures is desirable, a major limitation is the short time that the cultures represent dietary exposure and can be used before the cells differentiate or senesce and no longer are the cells of interest.

Use of immortalized cell lines has the disadvantage that the genetic alterations that stabilize the cell line for growth in culture may make it a poor model for the study of interest. However, the ability to culture these cells long term allows the investigator to design more comprehensive studies and collect...
more reproducible data. Immortalized cells can often be transplanted into an appropriate host and assessed both in vitro and in a live animal. However, use of a tumor cell line may provide little information on the effect of fatty acids on the development of the tumorigenic phenotype.

Genetically altered cells have the advantages and disadvantages of the immortalized cell lines noted above. However, such cell lines can be transfected with specific genes or have genes knocked out that may be involved in a pathway of interest. Thus, the effect of a particular fatty acid can be assessed in a known genetic background. Genetically altered cell lines have been used in few studies of nutrition and cancer. However, considering the importance of diet and genetic interactions, such models will be particularly important for future studies.

Cell culture conditions

The difficulty with cell culture is determining conditions that best represent the in vivo environment. Culture media are designed to improve cell growth, maintain a cellular phenotype, or promote a phenotypic change. Other than keeping the cells so that they maintain an appearance similar to their appearance in vivo, it is impossible to mimic the in vivo conditions. Cell culture conditions that will promote the needed growth and desired phenotype must be developed. It is desirable to use defined media, just as it is desirable to use defined ingredients in experimental diets. However, some cells do not grow well or will not maintain the desired phenotypic qualities in defined media.

The method of fatty acid addition to cultured cells can have a profound effect on the results of the experiment. The most widely accepted approach is to complex the fatty acids with albumin to deliver them to the cells in the aqueous-based media. In vivo fatty acids are present in complexes with serum lipoproteins and proteins, including albumin. Addition of fatty acids to serum will solubilize the fatty acids, but the serum component binding the fatty acids may not be known and considerable amounts of fatty acids are provided by serum. Defined media are recommended for studies involving supplementing cultured cells with fatty acids.

Selection of endpoints relevant to cancer

Cell culture studies are particularly useful in assessing mechanisms when conducted in conjunction with animal or human studies. Many of the hypothesized roles of dietary fatty acids in cancer center around their importance in cellular growth, proliferation, and apoptosis. Thus, endpoints assessed in cell culture experiments designed to probe the mechanism of fatty acid effects on carcinogenesis often focus on these areas of research. Cell culture studies can assess the effect of specific lipids on downstream events that are important for cellular responsiveness (32).

A related area of intense investigation with fatty acids and cultured cells is prostaglandin production. Prostaglandins are hormone-like compounds that have arachidonic acid (20:4n-6) as their precursor. Because essential fatty acids, particularly 18:2n-6, have been studied for their effect on tumor development and growth and because essential fatty acids are required to provide 20:4n-6, some of the effects of fatty acids on cancer development likely occur through alterations in the patterns of prostaglandin produced.

Dietary fatty acids may also modify hormonal secretion or the action of hormones on their target tissues. For example, animal and human studies have probed changes in circulating hormones with different amounts of dietary fat intake and different fatty acid dietary profiles (33). Controlled studies on cellular responsiveness to relevant hormone exposure can be conducted in target tissues or in cultured cells. Numerous endpoints have been assessed, including changes in cellular signaling, gene expression, and phosphorylation or dephosphorylation. Such investigations may clearly be relevant to the effects of fatty acids on cellular proliferation mentioned above.

Dietary fatty acids may also modulate immune function in a manner important to cancer development (34). For example, there is evidence of immune suppression by diets rich in polyunsaturated fatty acids. Because prostaglandins play a critical role in immune responsiveness and because dietary fatty acids influence prostaglandin metabolism, much research has focused on the influence of dietary fatty acids on macrophage-mediated inflammatory responses. Fatty acids also modify cytokine secretion, which may influence host-tumor interactions.

In vivo confirmation of mechanisms observed in vitro

In vitro systems can be useful in exploring the mechanistic basis for the effects of dietary fatty acid on cancer. However, the validity of the in vitro observations should be tested in an experimental animal or human system to verify that the in vitro observations are applicable to the in vivo models.

CONCLUSIONS

One difficulty with animal investigations is the different results observed when comparing a series of studies. Depending on design aspects, including dietary variables, the results of tumorigenesis studies can differ even when the same experimental model is used. This issue will be discussed in greater depth in other articles in this supplement in relation to models of breast and colon cancer. Researchers must continue to use a variety of models and dietary approaches to investigate different aspects of cancer in humans because no particular diet and model are relevant to the effects of fatty acids on human cancer. Furthermore, different models of cancer at a site can give entirely different results. As discussed above, pancreatic acinar carcinogenesis induced by azaserine was enhanced to a greater extent by lipids rich in unsaturated fatty acids than by those rich in saturated fatty acids (8). The azaserine-induced model contrasts with the nitrosamine-induced model, which results in ductular pancreatic carcinomas, where fats rich in saturated and unsaturated fatty acids were both effective in enhancing cancer (22).

Experimental studies are particularly useful for dissecting complex dietary interactions that cannot be separated in human investigations. For example, in investigations in humans it is difficult to discriminate between dietary variables such as high saturated fat intake and consumption of animal-based foods. Experimental studies can more effectively target the particular fatty acids of interest. Finally, animal and cell culture experiments are a powerful tool for establishing mechanisms for the effects of dietary fatty acids on cancer.
REFERENCES


