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MEDIUM-TERM PROTOCOLS FOR *IN VIVO* EVALUATION OF CHEMICAL MODIFIERS OF CARCINOGENESIS

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Cancer development is a long-term multistep process which allows interventional measure before the clinical disease emerges. The detection of natural substances which can block the process of carcinogenesis is as important as the identification of anti-tumoral drugs since they might be used in chemoprevention of cancer in high-risk groups. In vivo *rodent models of chemical carcinogenesis have been used to study plant-derived inhibitors of carcinogenesis such as indols, coumarins, isothiocyanates, flavones, phenols and allyl-sulfides. Since the standard* in vivo *rodent bioassay is prolonged and expensive, shorter reliable protocols are needed. Two* in vivo *medium-term protocols for evaluation of modifiers of carcinogenesis are presented, one related to liver and the other to bladder cancer. Both protocols use rats, last 8 and 36 weeks and are based on the two-step concept of carcinogenesis: initiation and promotion. The protocols use respectively the development of altered foci of hepatocytes expressing immunohistochemically the placental form of gluthation S-transferase and the appearence of pre-neoplastic urothelium and papillomas as the "end-points". The use of these protocols for detection of plant-derived inhibitors of carcinogenesis appear warranted.*

Key words: carcinogenesis – chemoprevention – bladder cancer – liver cancer – plant – derived inhibitors

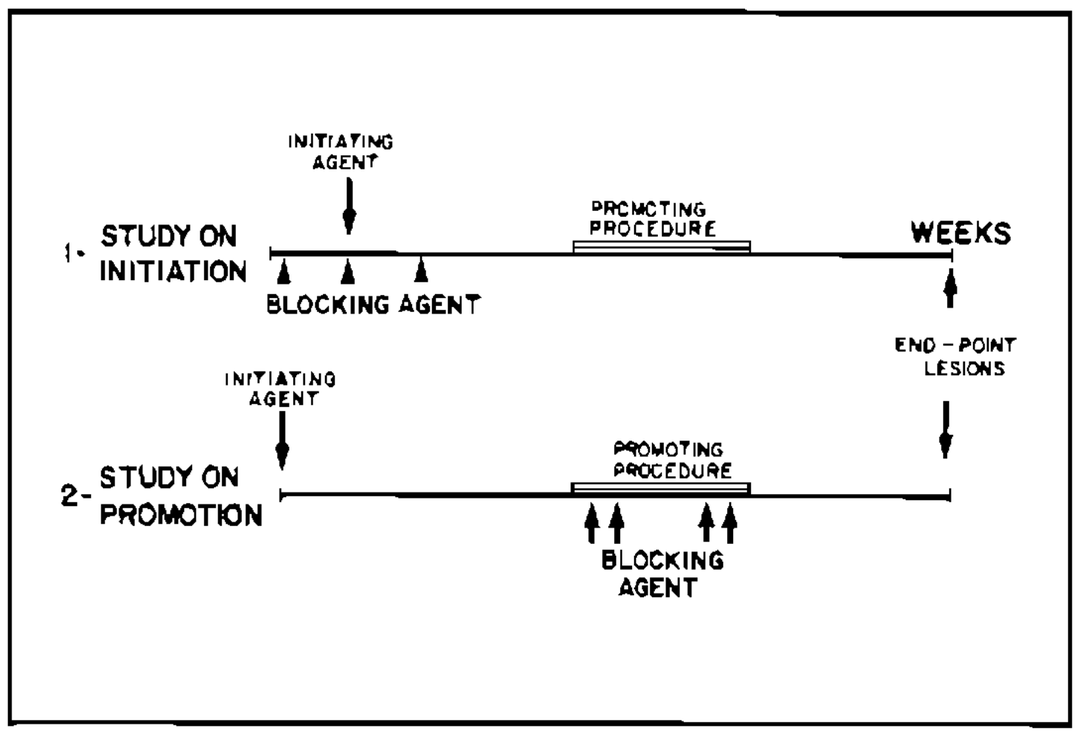
Cancer development is a long term process and experimental studies suggest that it occurs through different and sequential steps named initiation, promotion and progression (Faber, 1987, 1988; Weinstein, 1988; Pitot, 1989). Initiation is associated with a more or less permanent change in the phenotype of a target cell, presumably due to a change in DNA base composition or to gene rearrangements, induced by exposure to a carcinogen. Thus, initiating agents are genotoxic. Promotion is the process whereby initiated cells undergo focal proliferations, one or more of which may act as precursors for subsequent steps in carcinogenesis (Faber, 1987, 1988; Weinstein, 1988). Progression is the process whereby one or more focal proliferations undergo slow cellular evolution to malignant neoplasm. Progression is self generating but can be modulated by dietary procedures, drugs or xenobiotics (Pitot, 1989). It is biologically plausible to suggest that a similar pattern of cancer development occurs in humans although in this case evidence of exposure to initiating, promoting and modifying agents is not clear since during lifetime humans are submitted to a complex mixture of substances (Swanson, 1988). The prolonged nature of the promotion-progression process and its modulatability indicate that these stages are vulnerable sites for interventional measures to prevent the development of clinical disease (Belman, 1983; Weinstein, 1988; Farber, 1988).

Animal models are very convenient to identify chemical substances which can modify (induce or block) the process of carcinogenesis. In fact, one of the standard procedures to evaluate *in vivo* the carcinogenic potential of chemicals is the long-term assay with rodents (NTP, 1984; Moore, 1988). Mice and rats, under controlled conditions, are exposed during lifetime to a test substance; the rate of tumor incidence in the exposed animals, as compared to controls, points to a possible carcinogenic potential of the substance. This assay, however, is too long and expensive. Also, it does not take in consideration the multistage nature of carcinogenesis and does not permit previous knowledge of the organs that will eventually develop neoplasia. Nevertheless, with such a protocol it would be possible to evaluate the anti-carcinogenic effect of one substance if it were provided to the animals simultaneously to or before another known to be a definite carcinogen. An improvement in the study of modifying agents was the use of definite carcinogen with organ-specific carcinogenicity. Earlier studies by Wattenberg et als. on rodent

breast cancer and lung/forestomach cancer using respectively the organ-specific carcinogens dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene led to the recognition of the anti-carcinogenic property of indols and isothiocyanates (Wattenberg, 1977; Wattenberg & Loub, 1978). These are naturaly occurring substances in cruciferous vegetables like Brussels sprouts, cabbage, cauliflower and broccoli. Since in these early studies the protecting substances were provided to the animals just before or at the same time as the carcinogen they did exert their protective action by alterating the initiation step, probably by inducing the Phase I and/or II of the detoxifying sistems which led to biochemical transformations of the carcinogen.

The close relationship between results in animal assays and activity at human level is well demonstrated by studies on organic allyl sulfides (OAS), which are components of plants of the genus *Allium* (onions and garlic). Data obtained so far indicate inhibition by OAS of rodent chemically-induced carcinogenesis in six tissues, i.e., forestomach, lung, large bowel, esophagus, skin and liver. Carcinogens belonging to three chemical classes i.e., polycyclic aromatic hydrocarbons, hydrazynes and nitrosamines have been inhibited as well as tumor promotion (Belman, 1983; Hayes et al., 1987; Sparnins et al., 1988). It is not surprising that a recent epidemiological survey showed reduction of cancer risk paralel to increased consumption of allium vegetables. That was recently reported for gastric cancer in a high-risk area for this type of neoplasia in China. Protective effects were seen for garlic, onions and other allium foods (You et al., 1989). Therefore, experimental studies using *in vivo* rodent protocols are of value either for the better understanding of the balance of factors involved in the neoplastic response to environmental carcinogens as well as to identify potential naturally-derived chemoprotectors for cancer. Once identified, these anti-carcinogenic substances might be used for chemoprotection of high-risk groups like cancer-prone families, smokers, workers at chemical industries, handlers of substances with carcinogenic potential, etc... (Weinstein, 1988; Swanson, 1988; Lispin, 1988).

Another improvement in the design of *in vivo* assays for carcinogenesis was the recent development of protocols which allow to evaluate independently the action of substances either at the initiation or at the promotion/progression step (Shirai et al., 1987; Ito et al., 1988; Ward & Ito, 1988) (Figure). These protocols also allow to reduce the experimental duration in such a way that they can be classified as medium-term assays, i.e., bioassays shorter than the conventional two-year standard study. The relatively shorter period of time (weeks to months) save laboratory facilities, time and money (Ward & Ito, 1988).



General protocols for *in vivo* rodent chemoprevention studies based on the two-stage concept of carcinogenesis. Protocol 1: for studies on initiation. Protocol 2: for studies on promotion. The end-point lesions should be actual neoplasia or definite well-known preneoplastic alterations. Appropriate control groups should also be run.

Herein two medium-term bioassays designed to evaluate the modifying effects of chemicals on the rat liver and bladder carcinogenesis processes are presented. Their designs are presented in the Table. The shortage of time was accomplished by using specific procedures like partial hepatectomy or mechanical trauma to the urothelium by uracil cristals respectively in the liver foci assay and in the bladder cancer assay (Shirai et al., 1987; Ito et al., 1988). These procedures induce strong diffuse cellular proliferation, including of the carcinogen-initiated cells, which is an essential step for promotion of carcinogenesis (Colburn et al., 1987; Bogen, 1989).

Previous studies have validated these assays in such a way that foci of hepatocytes expressing the placental form of the enzyme glutatione S-transferase (GTS-P) were defined as precursors of the rat hepatocellular carcinoma (Sato et al., 1984), and urothelial lesions like nodular and papillary hiperplasia were established as actual

TABLE

Two *in vivo* medium-term rodent assays for detection of modifying agents of chemical carcinogenesis

	Liver foci assay (Ito et al., 1988)	Bladder cancer assay (Shirai et al., 1987)
Test animal	Rat	Rat
Duration of the assay	8 weeks	20 weeks
Initiating agent	Dietilnitrosamine (DEN) 200 mg/kg i.p.	Butyl-hidroxy-butylnitrosamine (BBN) 0,05% at the drinking water, 4 weeks
Enhacing procedure for cell proliferation	Partial hepatectomy, at week 2	Uracil salt, 3% in the diet, during 3 weeks
Test-substance	In the diet, from week 3 to 8	In the diet, from week 4 to 20, not during the three weeks on uracil
Assay end-point	Putative preneoplastic GST-P foci[a] of hepatocytes (quantitative evaluation)	Urothelial nodular or papillary hyperplasia, "papillomas", cancer (quantitative evaluation)
Control	For the initiating procedure and for the test substance separately	For the initiating procedure and for the test substance separately

a: GST-P foci = foci of hepatocytes which express immunohistochemically the enzyme glutathine-S-peroxidase, placental form.

preneoplastic lesions (Fukushima et al., 1982). Both assays demand for a well-trained pathologist and a system for image analysis. The results for the liver foci assay are expressed in number and size of foci per cm^2 of analysed liver; in the bladder assay the results are expressed in number of lesions per linear centimeter of urothelial basement membrane. The final report may be issued as late as 4 weeks after completion of the assay.

In the last decade the idea that cancer is an environmental disease became well established (Doll & Peto, 1981). This environmentalist view of cancer has emphasized the importance of prevention and early interventional measures (Doll & Peto, 1981; Weinstein, 1988; Farber, 1988; Swanson, 1988), besides the classical late therapeutic approach. Therefore, the detection of natural substances which can block the process of carcinogenesis is as important as the identification of antitumor drugs since the former might be used in chemoprevention of cancer in high-risk groups. The scientific community involved in evaluation of biological properties of natural substances should take advantage of protocols like those presented here.

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