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MODULATION OF HEPATOCARCINOGENESIS

*M. B. Roberfroid, N. Delzenne, and V. Préat**

Unité de Biochimie Toxicologique et Cancérologique, Department of Pharmaceutical Sciences, Université Catholique de Louvain, Brussels, Belgium

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INTRODUCTION

The administration of a wide variety of chemicals induces the long-term multiphase process that culminates in the appearance of liver cancer(s) (i.e. malignant growth of the tissue). Most of these procedures have been developed for mechanistic studies (1) and involve the following: a single (2–4) or several consecutive doses (e.g. the "stop model" of Bannasch and coworkers (5, 6)) of a so-called genotoxic carcinogen or initiator (the most commonly used compounds are nitrosamines, mycotoxins, and aromatic amines); a single high-dose initiating treatment of diethylnitrosamine or aflatoxin combined with several consecutive doses of a compound such as 2-acetylaminofluorene that in conjunction with a mitogenic hit, stimulate the focal development of putative resistant hepatocytes induced during initiation (the so-called resistant hepatocyte model developed by Solt & Farber (7)); the sequential administration of one or more doses of an initiator (a nitrosamine or an aromatic amine or amide) followed by long-term continuous exposure to a nongenotoxic carcinogen or promoter (the most commonly used liver cancer promoter is phenobarbital) (8, 9). In addition to short- or long-term exposure to one or more carcinogenic chemicals, some procedures also include, generally early in the process, a stimulus (e.g. partial hepatectomy

*V. Préat is Chercheur qualifié du Fonds National de la Recherche Scientifique, Belgium; her current address is Unité de Pharmacie Galénique, Department of Pharmaceutical Sciences, Université Catholique de Louvain, Brussels

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(10, 11) or a necrogenic dose of an hepatotoxin (7)) to induce cell proliferation in this highly differentiated but nonproliferating organ.

Within a few weeks or even days, these procedures all induce transitory states characterized by the appearance of foci of proliferation and/or tumors in the liver parenchyma (i.e. an abnormal mass of tissue whose growth exceeds and is uncoordinated with that of normal tissue and which persists unabated even after the stimuli have been withdrawn (12)). These foci or tumors are called (pre-)neoplastic lesions, enzyme- or phenotypically altered lesions, nodules, or adenomas, and are thought to be the precursors of cancer(s) (1, 5–7, 13–15). These transitory states are invaluable for studying the mechanisms of malignant transformation because they are landmarks in the development of cancer. Moreover, they are used as endpoints to screen for or to identify potential hepatocarcinogens (either *initiators* or *promotors*) in the in vivo short-term test for hepatocarcinogenicity (14). They also serve as markers for the early diagnosis of liver carcinogenesis. Our understanding of the mechanisms of early and midterm stages in liver carcinogenesis (15–18) has been greatly enhanced by the use of these tools and should improve even further in the near future as the techniques of molecular biology and cellular biochemistry are applied. Hence it is understandable that the morphological, biochemical as well as biological properties and the kinetics of development and evolution of these transitory states should have attracted so much scientific attention (18–20).

However, most of these procedures are also fully carcinogenic, i.e. they induce the appearance of histologically recognizable cancer(s), the real endpoint of carcinogenesis, in a large (up to 100%) proportion of animals within a relatively short period of time in most cases. Surprisingly few studies have investigated this aspect of liver carcinogenesis that includes not only the properties but most significantly the kinetics of appearance of cancer(s). The parameters for such studies are: *latency* of cancer development as measured by how long it takes after the first dose of the initiator for the first cancer to appear in treated animals; *incidence* of cancers as expressed by the percentage of treated animals contracting cancer(s); *yield* of cancers, which is the number of malignant tumors in animals that develop cancer(s); *histological nature* of liver cancers, a means of classifying chemically induced malignant tumors and characterizing their invasiveness.

THE CONCEPT OF MODULATION OF HEPATOCARCINOGENESIS

By focusing on the kinetics of incidence and yield of histologically characterized cancer (21, 22) in the liver (see below), we have formulated the hypothesis that a *fully hepatocarcinogenic process* (i.e. self-sustaining up until there is a significant incidence and yield of liver cancer(s) without any

further procedure) can still be either positively or negatively modulated. In this hypothesis, *modulation* results from any procedure or experimental condition that, while not itself a prerequisite for cancer, has the following effects: is able either to shorten or lengthen the latent period; increase or decrease the incidence and/or yield of cancers; and/or modify the histological and the invasive nature of cancers (i.e. the extent of metastasis). Table 1 summarizes the criteria for determining positive or negative modulators of carcinogenesis.

We stress that this terminology, which has already been used in the scientific literature on chemical carcinogenesis but has not yet been clearly defined, does not in any way contradict or restrict the more conventional term *promotion*. Promotion is indeed a well-established and highly valuable concept (1, 18, 19) to characterize the effect of exposure to nongenotoxic chemicals that are often essential to complete the action of a low carcinogenic-genotoxic initiator. The demonstration of a promotor's effect on hepatocarcinogenesis can be and often is limited to increased incidence and/or accelerated appearance of foci of cell proliferation and/or benign tumors (19, 20). *Modulation,* as defined in this review, has no direct relevance to multiphase/multistep carcinogenesis. Its effect can only be demonstrated by following the kinetics of cancers as they appear and/or by recording the incidence and yield of histologically characterized cancers. We propose this term hoping that it will broaden our view on carcinogenesis, particularly regarding the effects of more systemic procedures or experimental conditions such as chronic exposure to chemicals, dietary imbalances, or surgery that disrupts metabolic or proliferative homeostasis. Even though such procedures are not essential for making an otherwise induced process fully carcinogenic, they can still influence its pathogenesis by creating conditions that speed up or retard the kinetics of its development to malignancy and consequently increase or decrease the incidence and/or yield of cancer.

This review aims to present and discuss first our own experimental findings over the past 10 years, from which the concept of modulation has been derived; and second, supporting experimental data reported by others from

Table 1 Summary of the effects of positive and negative modulation on neoplastic development*

Criteria to evaluate modulation of cancer	Nature of modulation	
	Positive	Negative
Latent period	shorter	longer
Kinetics of formation	speeding up	slowing down
Incidence	higher	lower
Yield	higher	lower
Invasiveness	= or higher	= or lower

*One effect is sufficient to determine modulation

experiments that were not necessarily related. The reader is referred to earlier articles (23–28) to trace the progression of our thinking in this subject over the past 3–4 years.

ORIGIN OF THE CONCEPT OF MODULATION OF HEPATOCARCINOGENESIS

Among the various procedures known to be fully hepatocarcinogenic we have chosen the resistant hepatocyte model developed by Solt & Farber (7). Under our experimental conditions (29, 30) male Wistar rats received the treatment described in the legend of Figure 1. The incidence of hepatocellular carcinoma in these animals (IS) reached 80% 13 months after initiation; the latent period before the first cancer appeared was in the order of 6 months; thereafter the incidence of cancer increased almost linearly with time and the mean yield never exceeded 1–1.5 cancers per rat contracting cancer (Figure 1) (31). However, if starting at week 5 after initiation, the rats were fed a diet containing phenobarbital (IS PB) or the peroxisome proliferator nafenopin (IS NAF), not only was the latent period shortened but, more significantly, the kinetics of increase in incidence and yield of cancers was dramatically accelerated. In phenobarbital-treated rats the incidence already reached 65% at month 6. In nafenopin-treated rats, the incidence increased even faster since 85% and 90% of treated animals had cancers after 5 and 6 months, respectively (Figure 1; 31, 32). In these experimental conditions, increased incidence was accompanied by increased yield. Indeed, phenobarbital-fed rats already had 2 cancers per cancer-contracting rat after 6 months, a figure that increased linearly with time up to 8.5 at month 13. In nafenopin-fed rats the increase in yield was even more dramatic, reaching a mean value of 7.5 cancers per cancer-contracting rat after 6 months (Figure 1; 31, 32). In the case of exposure to phenobarbital, delay in administration of the drug for at least 3 months still caused an increase in cancer incidence provided that the drug was given for a period of at least 3 months prior to another 3 months of feeding basal diet (33). Note that under these experimental conditions, no correlation was found between latent period, incidence, or yield of cancers and latent period, incidence, or yield of foci or nodules preceding the appearance of cancers. In particular, although the latent period was shorter and the kinetics of incidence and yield of cancers faster, the nafenopin-fed rats (IS NAF) always displayed a lower yield of phenotypically altered foci and nodules even when the rare lesions that occurred had some of the histological characteristics of highly cancer-prone lesions (6).

Our hypothesis on the concept of modulation of hepatocarcinogenesis rests on these data. Indeed the procedure (IS) of the resistant hepatocyte model was shown to be fully carcinogenic since it induced cancers in a significant

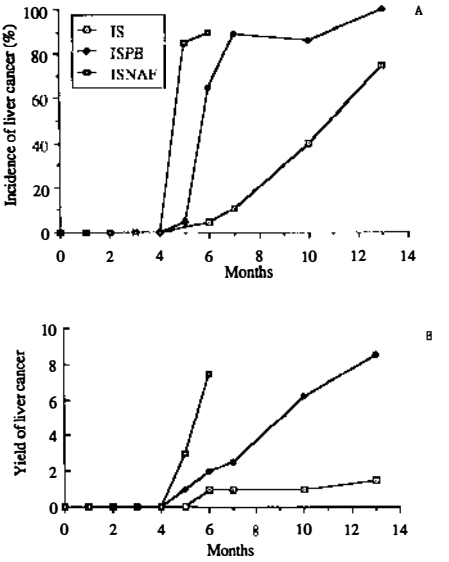

Figure 1 Evolution of cancer incidence (A) and yield (B) in rats undergoing the fully carcinogenic procedure of the resistant hepatocyte model and the effect of modulation with a phenobarbital- and nafenopin-containing diet. Male Wistar rats were initiated (I) with a single high dose of diethylnitrosamine (200 mg/kg ip) followed 2 weeks later by a selection (S) consisting of feeding a diet containing 0.03% 2-acetylaminofluorene for 2 weeks and one necrogenic dose of CCl_4 (2 mg/kg po) at day 7. Five weeks after I, rats received either a basal diet (IS), or a diet containing phenobarbital (0.05%) (IS PB) or nafenopin (0.1%) (IS NAF). (For details, see refs. 31, 32)

percentage of treated animals within ± year. Feeding a diet containing phenobarbital (IS PB) or nafenopin (IS NAF) positively modulated this hepatocarcinogenic process by shortening the latent period and increasing incidence and yield of histologically recognizable cancers.

Since modulation of hepatocarcinogenesis affected the kinetics of cancer development, we also hypothesized that recording cancer incidence and yield at a particular time point (e.g. 6 months) could be used to identify, but not to compare the potency of, potential modulating treatments or experimental conditions. Table 2 shows that by using such an approach, feeding rats diets containing the psychotropic drug oxazepam (34), the peroxisome proliferator perfluorooctanoate (35), or the pesticides DDT (32), and 2,4,5, trichlorophenoxyacetic acid (35) positively modulated hepatocarcinogenesis by increasing IS-induced cancer incidence and (but only for DDT) yield, 6 months after initiation by diethylnitrosamine. None of these treatments had any effect on the nature of cancers appearing after 6 months.

Table 2 Modulation of hepatocarcinogenesis by the chronic administration of various chemicals added in diet at the indicated concentration

Treatment	Number of rats	Cancer Incidence[a]	Cancer Yield[a]
IS[b]	70	5%	1
Oxazepam 0.05%	10	66%	1
Perfluorooctanoate 0.015%	10	33%	1
DDT 0.05%	10	66%	3
2,4,5-T 0.05%	10	20%	1

[a] Incidence and yield of cancer(s) were measured 6 months after initiation by diethylnitrosamine in male Wistar rats submitted to a IS protocol.
[b] See legend of Figure 1.

In addition to the continuous administration of various chemicals, surgery was also performed on the rats in the fully carcinogenic experiment (IS). Surgical procedures included: the classical portacaval shunt (PCS) in which the liver receives no venous blood supply; the so-called false mesentericocaval shunt (fMCS) that limits liver venous blood supply to the pancreaticoduodenal vein; the portacaval transposition (PCT) in which the liver receives the blood from the veina cava but not from portal, splenic, or pancreaticoduodenal veins; and finally, the true mesentericocaval shunt (MCS) that restricts liver venous blood supply through splenic and pancreaticoduodenal veins excluding mesenteric blood (Figure 2). These surgical interventions all modify the supply of hormones, nutrients and/or venous blood to the liver and are thus likely to cause changes in liver metabolic homeostasis. As shown in Figure 2, by their effect on cancer incidence 6 months after initiation, with the exception of the mesentericocaval shunt (MCS), which preserves most of the hormonal supply to the liver, all such surgical procedures, positively modulated the hepatocarcinogenic process induced by the resistant hepatocyte model (IS) (36–38; J. C. Pector, personal communication). A two-thirds partial hepatectomy was also performed either eight weeks before injecting diethylnitroamine (39) or one week after the last exposure to 2-acetylaminofluorene in the selection phase (37). Even though the long-term effects of such surgical interventions are not known, the short-term effect is liver cell proliferation. In both experimental conditions, the two-thirds partial hepatectomy positively modulated hepatocarcinogenesis by increasing cancer incidence in IS-treated rats six months after initiation (see Figure 1 for details of the protocol) from 5% to 30% or 70% in animals hepatectomized before or after IS, respectively (37, 39).

Our own accrued experimental data thus support the hypothesis of positive modulation of the pathogenic process induced by a fully hepatocarcinogenic procedure. Modulation could result from the effect of either the chronic

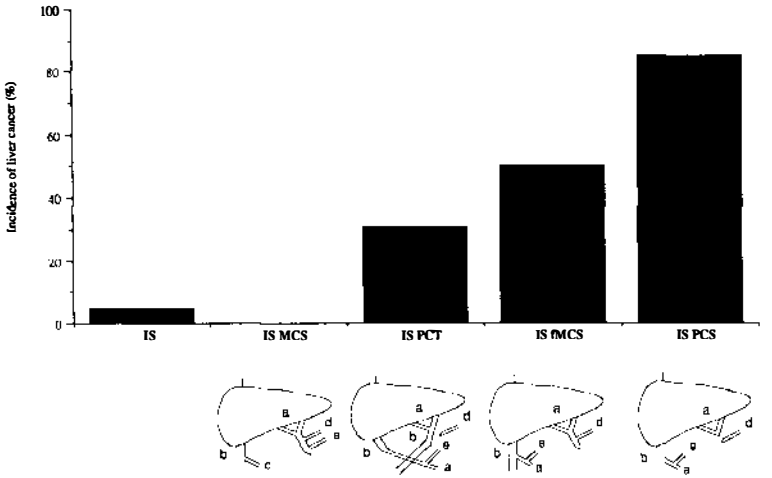

Figure 2 Evolution of cancer incidence in rats subjected to the fully carcinogenic procedure (IS) of the resistant hepatocyte model and the effect of various portal derivations. For details on IS protocol see legend of Figure 1. All surgical procedures were performed one week after stopping 2 acetylaminofluorene feeding. (see text for details; refs. 36–38). a = portal vein; b = inferior vein cava; c = mesenteric vein; d = pancreaticoduodenal vein; e = splenic vein.

administration of chemicals or of a surgical procedure disruptive of metabolic or proliferative homeostasis in the liver.

ILLUSTRATION OF THE CONCEPT OF MODULATION OF HEPATOCARCINOGENESIS

We now review other supporting experimental evidence, even though these results were not necessarily derived from experiments designed to test our hypothesis.

Modulation of Hepatocarcinogenesis by Continuous Exposure to Chemicals

Phenobarbital is the most extensively studied of the chemical compounds that promote hepatocarcinogenesis when given on a continuous basis to previously initiated rodents. It is even used as the positive control in many experiments. Although most of the studies reported in the literature are limited to the effect of phenobarbital on the appearance of foci or benign tumors (8, 14, 18) and thus beyond the scope of this review, nevertheless data have been published that support the concept of modulation of hepatocarcinogenesis by this drug.

Compared to the effect of an otherwise fully carcinogenic procedure followed by basal diet, long-term continuous feeding of a phenobarbital-containing diet increased both the incidence (40–42) and the yield (9, 42, 43) of hepatocellular carcinomas at a particular timepoint; it also modified the kinetics of the pathogenic process by accelerating the evolution to malignancy; and finally it decreased the mean survival time of animals dying from cancer (41, 44). Pereira et al (42), using diethylnitrosamine-treated mice, showed that modulation by long-term continuous exposure to phenobarbital depends on physiological factors. This treatment (see Table 1 for definition) negatively modulated hepatocarcinogenesis when given to young mice whereas it positively modulated it when given to adult animals. Besides phenobarbital, other chemicals were administered to rats or mice that had previously been subjected to an otherwise fully carcinogenic procedure (induced by nitrosamine or aromatic amine). Many of these chemicals positively modulate hepatocarcinogenesis under such experimental conditions (See Table 3).

In addition to positive modulation of hepatocarcinogenesis, some findings also support the concept of negative *modulation* by long-term continuous exposure to some chemicals. Masui et al (51) showed that sustained administration of ethoxyquin or diaminodiphenylmethane after IS treatment decreased the incidence and yield of hepatocellular carcinoma. Similarly, Weber et al (52) have reported data supporting the hypothesis that dehydroepiandrosterone may negatively modulate liver carcinogenesis induced by the procedure of the "stop model" (6); this compound had a rather qualitative effect. In fact it induced the appearance of cancers with a higher degree

Table 3 List of chemical procedures causing positive modulation of a fully hepatocarcinogenic process

Carcinogenic treatment	Chemical modulator	Reference
DEN[a]	2-hexa-chlorocyclohexane	45
DEN[a]	DDT	44
DEN[a]	CCl_4	46
DEN[a]	clofibrate	47
DEN[a]	WY-14,643	47
EHEN[b]	PCB[c]	48
3MeDAB	CCl_4	49
NNM[d]	estradiol phenylpropionate estradiol benzoate	50

[a] DEN: diethylnitrosamine
[b] EHEN: methylhydroxyethylnitrosamine
[c] MeDAB: methyldiaminobenzidine
[d] NNM: N-nitrosomorpholine
[e] PCB: polychlorinated biphenyl

of differentiation, a lower mitotic rate, and a reduced potential for metastasis as compared to those induced by the 7-week treatment with N-nitrosomorpholine alone.

Dietary Modulation of Hepatocarcinogenesis

Both quantitative as well as qualitative changes in diet fed to an animal otherwise subject to a fully carcinogenic procedure have been reported to modulate hepatocarcinogenesis (53). Lagopoulos et al (54) showed that a 30% reduction of daily calorie intake slowed the kinetics of development of hepatocellular carcinoma induced in mice by diethylnitrosamine. This treatment reduced the incidence of cancer to 0%, compared to mice fed ad libitum. At the same time point (36 weeks after initiation), these mice displayed a 100% incidence of the same cancer. According to Tannenbaum (55), negative modulation of hepatocarcinogenesis by dietary calorie restriction could be due to a decreased gain in body weight rather than to calorie restriction itself. Indeed this author has shown that decreasing body weight gain by other means (e.g. by increasing the basal metabolism, by lowering body temperature or by giving dinitrophenol) also resulted in a decreased tumor incidence despite comparable food intake.

In addition to this kind of negative dietary modulation due to reduced calorie intake or increased rate of basal metabolism, qualitative rather than quantitative changes in dietary intake can also modulate hepatocarcinogenesis. Enzmann et al (56) reported that adding fructose to the drinking-water (120g/l) of rats previously subject to the procedure of the "stop model", increased the incidence of hepatocellular carcinoma as well as the incidence of liver metastasis in the lung. Similarly, diets deficient in lipotropes and, in particular, choline-deficient diets given continuously to rats previously subjected to a fully carcinogenic procedure, positively modulated hepatocarcinogenesis as evidenced by increasing the incidence of hepatocellular carcinoma in comparison to rats receiving a normal diet during the same period (57). Rogers et al (58, 59) have also reported data showing that a lipotrope-deficient high-fat diet strongly enhanced the incidence of malignant liver tumors and decreased cancer mortality. By contrast, supplementing diet with methionine (a lipotrope compound) qualitatively modulated liver carcinogenesis by lowering the incidence of lung metastasis in rats subjected to diethylnitrosamine and phenobarbital or DDT (4).

Modulation of Hepatocarcinogenesis by Infestation

Thamavit et al (60) have shown that infestation by *Opisthorchis viverini* prior to induction of hepatocarcinogenesis by N-nitrosomorpholine strongly increased the incidence of cholangiocarcinoma in hamster.

SUMMARY, CONCLUSIONS, AND PERSPECTIVES

A wide variety of chemical procedures induce long-term multistage hepatocarcinogenesis in the rodent liver. Many of these procedures are short in duration but are nevertheless fully carcinogenic, i.e. they induce histologically recognizable cancer(s) in a large proportion of animals within a relatively short period of time. Most research on hepatocarcinogenesis has until now focused on the so-called foci and nodules, the transitory states that are probable landmarks in cancer development. These studies were aimed at understanding the mechanism(s) of liver cell transformation. But little emphasis has been placed on the kinetics of cancer appearance in the liver of animals subjected to such procedures or on the experimental conditions that could modify these kinetics.

We propose that neoplastic development as quantified by the incidence and yield of cancer(s) can be modulated. In this review we have discussed procedures or experimental conditions that, while not essential for neoplastic development and/or cancer appearance in the liver, can either shorten or lengthen the latent period preceding the appearance of; and/or either increase or decrease the incidence and/or yield of; and/or modify the invasiveness of cancers. These procedures and experimental conditions are said to cause positive or negative modulation of hepatocarcinogenesis, respectively. They can be performed on experimental animals undergoing a fully carcinogenic process by the following: long-term exposure to chemicals such as microsomal enzymes inducers, peroxisome proliferators, pesticides, etc; surgical modifications to liver blood supply or liver cell proliferation; changes in dietary intake; or liver infestation.

As far as the mechanism of modulation of cancer development in the liver is concerned, we hypothesize that it may result from changes in metabolic and/or proliferative homeostasis (27). Such changes would create conditions within the organ in which neoplastic development has otherwise been initiated and/or promoted that either facilitate or hinder the appearance of cancer, as evidenced by changes in incidence and/or yield as well as invasiveness. Indeed, modulating chemical procedures are known to cause pleiotropic metabolic effects (61); changes in liver blood supply markedly affect the supply of hormones and nutrients and thus cause metabolic adaptations; infestation frequently interferes with cellular homeostasis; and finally, changes in dietary intake are a classical means of influencing liver function. In this respect, it is particularly important to emphasize that calorie restriction appears to be a good experimental condition to negatively modulate hepatocarcinogenesis. Calorie restriction is also known to affect many different types of carcinogenic processes that are both spontaneous and chemically induced (62). It has been hypothesized that such changes in calorie intake may restore hormonal equilibrium and reinforce cellular homeostasis (28).

Chen et al (63) have recently provided solid experimental evidence to support the hypothesis that chronic energy intake restriction may reduce the expression of putative protooncogenes in mammary glands as well as in liver. This observation, if confirmed, could open new avenues in cancer research in which the concept of modulation of carcinogenesis could become important. It could be a new way to look at and give scientific support to strategies for cancer prevention.

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