Review

Hormesis in Carcinogenicity of Non-genotoxic Carcinogens

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Abstract: Recently the idea of hormesis, a biphasic dose-response relationship in which a chemical exerts opposite effects dependent on the dose, has attracted interest in the field of carcinogenesis. With non-genotoxic agents there is considerable experimental evidence in support of hormesis and the present review highlights current knowledge of dose-response effects. In particular, several in vivo studies have provided support for the idea that non-genotoxic carcinogens may inhibit hepatocarcinogenesis at low doses. Here, we survey the examples and discuss possible mechanisms of hormesis with cytochrome P450 inducers, such as phenobarbital, 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT), α-benzene hexachloride (α-BHC), and other non-genotoxins. Epigenetic processes differentially can be affected by agents that impinge on oxidative stress, DNA repair, cell proliferation, apoptosis, intracellular communication and cell signaling. Non-genotoxic carcinogens may target nuclear receptors, cause aberrant DNA methylation at the genomic level and induce post-translational modifications at the protein level, thereby impacting on the stability or activity of key regulatory proteins, including oncoproteins and tumor suppressor proteins. Via multiple epigenetic lesions, non-genotoxic carcinogens can elicit a variety of changes contributing to cellular carcinogenesis. (J Toxicol Pathol 2006; 19: 111–122)

Key words: hormesis, non-genotoxic carcinogen, threshold, oxidative stress

Exposure to Carcinogens and Human Cancer

The risk of cancer in humans is dependent on environmental, occupational and recreational exposure to carcinogens as well as on spontaneous events that reflect human variation in the efficiency or fidelity of various cancer-critical processes. Assessment of carcinogenic potential of agents to which human beings are exposed is clearly of prime importance but this is complicated by the existence of both genotoxic and non-genotoxic classes of chemical carcinogens, divided on the basis of their ability to react with DNA and form adducts. It is well established that genotoxic agents can covalently bind to DNA and increase the number of mutations, thereby causing errors in DNA replication. Positive data for chromosomal effects like aneugenicity or clastogenicity, in the absence of mutagenicity, may support separate characterization of compounds that exert carcinogenic effects only at high doses1. Non-DNA-reactive compounds, such as topoisomerase inhibitors2,3 or inhibitors of the spindle apparatus or associated motor proteins4–7 are considered to act by this mechanism8.

Many chemicals that produce tumors in experimental animals have been shown to act by epigenetic mechanisms that do not necessarily involve DNA attack or hereditable genetic alteration9. The indirect nature of the mechanisms involved means that prolonged exposure to high levels of chemicals is necessary for the production of tumors10. With such non-genotoxic carcinogens, theoretically cancer would not occur at exposures below a threshold at which the relevant cellular effect is not operative. Also, in contrast to DNA-reactive genotoxic effects, epigenetic mechanisms may be unique to the rodent species used for testing. Certain chemical carcinogens have been well studied and provide examples for the use of mechanistic information in risk assessment. Non-genotoxic carcinogens including tumor promoters, like dioxin for example, do not bind directly to DNA but alter cell proliferation and physiology by inducing expression of enzymes involved in xenobiotic metabolism, DNA repair, methylation and cell signaling. An altered hormonal environment may enhance the rate of cell replication by mechanisms involving receptor-mediated processes without DNA-reactivity, thus increasing the likelihood of promotion/progression of spontaneously initiated cells11.
Threshold in Carcinogenicity of Environmental Carcinogens

With examination of the risk of human exposure to chemicals with carcinogenic potential in the environment, a natural question is whether a threshold exists for observed effects. Recently the concepts of “practical” and “perfect” thresholds for genotoxic and non-genotoxic compounds, respectively, have been proposed. The idea is that carcinogens can be further classified as: (i) Genotoxic agents without a threshold in their effects; (ii) Genotoxic compounds for which the existence of a threshold is possible but is not yet sufficiently supported; (iii) Genotoxic carcinogens for which a “practical” threshold is supported by studies on mechanisms and/or toxicokinetics; (iv) Genotoxic carcinogens for which a “perfect” threshold is associated with a no-observed effect level (NOEL) and (v) Non-genotoxic carcinogens for which a “perfect” threshold is associated with a NOEL.

Until recently, risk assessment in the field of chemicals distinguished between two types of agents: the first comprising potentially toxic chemicals that may induce physical damage to human beings at above a certain threshold of exposure or intake. The second class is believed to cause harm at any level above zero, even at very tiny doses (stochastic effects). However, the conventional view of toxicity and risk has been challenged by recent investigations pointing to potential beneficial effects of exposure to otherwise hazardous substances at very low dose levels. Most of the substances involved are non-genotoxic chemicals, acting as cytochrome P-450 inducers at high doses and exhibiting promoting effects on hepatocarcinogenesis in rodents, and the existence of a threshold was postulated for examples acting by epigenetic mechanisms, such as phenobarbital, α-benzene hexachloride (α-BHC), 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT) and trichloroethylene (TCE). However, genotoxic carcinogens, such as 2-acetylaminofluorene (2-AAF) and α-phenylnapthalene, and ionizing radiation may also be included. Inhibitory effects of all these agents on carcinogenesis at low doses have been subsumed under the heading of hormesis.

The Hormesis Theory

Hormesis has been defined as a dose/response relationship in which there is a biological activation at low doses, but an inhibition at high doses, or vice versa, resulting in a U, J or inverted-U shaped dose response.

The history of hormesis originated in the laboratory of Prof. Hugo Schulz at the University of Greifswald in Northern Germany. He found that many agents appeared to stimulate metabolism at low concentrations but inhibit them at higher doses. This provided a toxicological explanation for his development of homeopathic ideas. Interest in the effects of low doses rapidly expanded, especially with many studies of interactions involving (mainly) plants, bacteria and fungi, most notably in Europe, the USA and Japan. Hormetic effects were observed at low exposure levels based on the dose-response pattern with data from developmental toxicity studies, indicating that there might actually be a reduced risk of toxic effects at low exposure levels.

Hormesis implies the existence of a threshold dose level and there are dose-response models that include parameters that account for the threshold.

With ionizing radiation, hormesis was interpreted to be due to adaptation to background radiation exposure, as well as metabolic protection against the array of other abiotic stresses in the environment. Weak endogenous carcinogens, such as reactive oxygen species (ROS), as well as micronutrient deficiencies and environmental toxins are obvious causes of non-radiation-induced DNA damage which might lead to oncogenic transformation in non-irradiated cells. The results suggested that at the level of background radiation, various forms of non-radiation DNA damage in tissues occur to much higher extents than those due to the low-dose radiation exposure. It has been proposed from the published data that mammalian cells have the physiological capacity to protect themselves constantly by preventing and repairing DNA damage. Furthermore, damaged cells are susceptible to removal by apoptosis or the immune system and chronic low-dose-rate radiation activate the immune system of the whole body. Low-dose radiation was suggested to induce cellular signaling that may stimulate cellular protection systems over hours to weeks. Enhanced and persistent protective responses might reduce the steady state level of non-radiation DNA damage, thereby impacting on deleterious outcomes such as cancer and aging.

Hormetic Effects in Carcinogenesis

The question whether the concept of hormesis can be generalized to carcinogenesis has been recently discussed. The history of hormesis originated in the laboratory of Prof. Hugo Schulz at the University of Greifswald in Northern Germany. He found that many agents appeared to stimulate metabolism at low concentrations but inhibit them at higher doses. This provided a toxicological explanation for his development of homeopathic ideas. Interest in the effects of low doses rapidly expanded, especially with many studies of interactions involving (mainly) plants, bacteria and fungi, most notably in Europe, the USA and Japan. Hormetic effects were observed at low exposure levels based on the dose-response pattern with data from developmental toxicity studies, indicating that there might actually be a reduced risk of toxic effects at low exposure levels.

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assessment of the effects of Hg$^{2+}$ on O$_6$-methylguanine-DNA methyltransferase activity of human buccal fibroblasts by Liu et al.$^{32}$ revealed elevation at low doses of 0.3 to 3 µM. With the dose-response curves of rat hepatic DNA damage for different types of carcinogens assessed by Kitchen and Brown$^{33}$, 11 showed non-monotonic character with some treated values lower than in controls.

The promotion stage of carcinogenesis has also been studied in the low dose range with regard to various parameters of interest. Examples include cell turnover with caffeic acid in the rat forestomach and kidney, altered hepatic foci formation with TCDD in diethylnitrosamine (DEN)-pretreated partially hepatectomized rats$^{17}$, and urinary bladder hyperplasia in saccharin-treated rats$^{34}$. Several chronic bioassays for carcinogenicity in rats and mice have demonstrated a negative correlation between proliferative hepatocellular lesions and lymphomas at low and medium dose levels$^{15}$. In addition, TCDD at hepatocarcinogenic doses was reported to be capable of causing dose-dependent reduction in mammary and uterine tumors$^{36}$. In 1994, Cook$^{37}$ reported that dioxin-treated rats displayed substantial decrease in tumors of the adrenals and pancreas and more modestly, in the liver. Examples of hormesis also include TCDD-mediated reduction in tumor incidence after exposure to low doses of radiation$^{20}$ or metals such as selenium$^{38}$. U-shape responses were also observed for chemically induced pulmonary tumors$^{39–41}$ and testicular cancer$^{42}$.

**Threshold in Phenobarbital Hepatocarcinogenicity**

Recently, especial attention has been devoted to the carcinogenicity of low doses of phenobarbital, a sedative and anticolulsant, which is used widely for long-term clinical therapy. It is also a well-known non-genotoxic carcinogen and tumor promoter in rodents. Epidemiological studies have not shown phenobarbital-related tumors in humans, indicating that humans may have low sensitivity to toxic effects of phenobarbital. In the rat, Goldsworthy et al.$^{43}$ reported no promotion by phenobarbital below 10 ppm with regard to the enzyme-altered foci. Furthermore, Kitagawa et al.$^{44}$ found inhibitory effects of both phenobarbital and another tumor promoter, DDT, on carcinogenesis when given together with relatively high doses of carcinogens. Similarly, Pitot et al.$^{45}$ found a slight decrease of altered hepatic foci by 10 ppm phenobarbital, and Maekawa et al.$^{46}$ demonstrated similar results with 1 ppm phenobarbital. To determine the practical threshold level for hepatopromoting effects of phenobarbital, Kitano et al.$^{13}$ investigated dose dependence using a rat liver medium-term bioassay (Ito test)$^{47}$. When phenobarbital was administered to rats in a wide range of doses of 0.01 to 500 ppm in the diet for 6 weeks after a single intraperitoneal injection of DEN in serial experiments, glutathione S-transferase placental form (GST-P) positive foci, preneoplastic lesions in the liver, were found to be increased dose dependently in rats given 60–500 ppm. However, with doses in the range of 1–7.5 ppm, decrease was evident as compared to the control group, this being statistically significant at 1 and 2 ppm (Fig. 1). It was concluded that phenobarbital effects reflect hormesis in the rat liver, indicating the existence of a threshold for its carcinogenicity, suggested to be related to inhibition of cytochrome P-450 CYP3A2 protein expression by low doses of the chemical$^{13}$.

For further clarification of the hormetic influence of
phenobarbital, Kinoshita et al. investigated doses of 0, 2, 15 and 500 ppm applied in diet to male F344 rats for 10 or 33 weeks after initiation of hepatocarcinogenesis with DEN. Formation of GST-P positive foci and liver tumors was inhibited at 2 ppm after 10 and 33 weeks of phenobarbital administration, respectively (Fig. 2). Histopathological examination further demonstrated a significant reduction in the multiplicity of total tumors, in particular, hepatocellular carcinomas (HCCs), and a tendency for decreased incidences of HCCs and adenomas at 2 ppm14. In contrast, high dose administration resulted in strong elevation of HCC and total tumor multiplicities, this appearing to be related to increased generation of hydroxyl radicals, a marker of oxidative damage 8-OHdG, CYP2B1/2 and CYP3A2 mRNAs and the protein level, activity, and gene expression of other Phase I and II xenobiotic metabolizing enzymes. Inhibition at low doses was considered to be due to the suppression of 8-OHdG generation and cellular proliferation within areas of GST-P positive foci, as well as programmed cell death, apoptosis, in background liver parenchyma. The decrease of 8-OHdG levels induced by phenobarbital at low dose was possibly a result of elevated expression of the gene encoding the enzyme responsible for the repair of 8-OHdG lesions, oxoguanine glycosylase 1 (Ogg1). The reduction of apoptosis in the normal-appearing liver tissue surrounding the GST-P positive foci, which might have been due to the inhibition of oxidative DNA damage, was suggested to suppress enlargement of foci because of elevated sensitivity to stimuli for regeneration14. Another explanation for the suppressive effect of phenobarbital on the development of preneoplastic lesions might involve stimulation of hepatic drug-metabolizing enzymes, which detoxify carcinogens45. Activation of P-450 isoenzymes CYP2C11 and NADPH-cytochrome P-450 reductase (OR) in liver microsomes observed after administration of phenobarbital at low dose, if not accompanied by elevation of their protein expression leading to the generation of large amount of hydroxyl radical OH, might have a protective effect14. The available results thus indicate that the compound exhibits hormetic effects on rat hepatocarcinogenesis initiated with DEN by differentially altering cell proliferation, apoptosis and oxidative DNA damage at high and low doses.

Dose-response for α-benzene hexachloride hepatocarcinogenicity

α-BHC, a major organochlorine byproduct in the manufacture of lindane (γ-BHC), has been used in admixtures with lindane for agricultural purposes. The α-isomer of BHC has been categorized as a non-genotoxic carcinogen because of induction of liver tumors in rodents after high dose administration in the long-term but no mutagenicity in the Ames test. 2,4,6-trichlorophenol is the major metabolite in α-BHC metabolism by the cytochrome P-450 oxidoreductase system. After dechlorination and dehydrochlorination of α-BHC, removable chlorine atoms might react with hydrogen peroxide to produce hypochlorous radicals binding to DNA and formation of chlorinated DNA adducts, like 8-chloro-2-deoxyguanosine, 5-chloro-2-deoxycytidine and 8-chloro-2-deoxyadenosine48,49. Hyperplastic nodules and carcinomas in the livers of rats and mice were found induced by the long-term administration of high doses of α-BHC (such as 500 or 1000 ppm), but not β- and γ-BHC50,51. Furthermore, early toxicological studies revealed that α-, β-, and γ-BHC are potent inducers of hepatic monoxygenases in rats52, in addition to causing liver enlargement53,54. Since induction of the monoxygenase system is assumed to influence the promotion stage55,56, the mechanism of α-BHC carcinogenicity is likely to be due to its influence on spontaneously initiated hepatocytes50,51.

To search whether α-BHC exhibits hormesis regarding...
its hepatocarcinogenicity in rodents the dose dependence of its promoting effects was first investigated by Masuda et al.15 in a medium-term rat liver bioassay (Ito test). When F344 male rats were given α-BHC at a wide range of doses from 0.01 to 500 ppm in the diet for 6 weeks after a single intraperitoneal injection of DEN, quantitative values for numbers and areas of GST-P positive foci were dose-dependently increased at 0.5 to 500 ppm. However, a tendency for a decrease was observed with 0.01 and 0.1 ppm α-BHC (Fig. 3). As observed with phenobarbital, CYP3A2 protein levels and activities showed a good correlation with the numbers and areas of GST-P positive foci. This experiment provided supportive evidence for hormesis in the promotion by α-BHC of rat hepatocarcinogenesis and suggested that the mechanism might be related to the suppression of P-450 isoenzyme CYP3A2 protein expression by low doses15.

A second study was conducted with α-BHC applied to F344 rats at doses of 0.01 to 500 ppm for 10 weeks after DEN initiation57. While α-BHC promoted the formation of GST-P positive foci at the dose of 500 ppm, both the numbers and areas of preneoplastic lesions were found to be significantly reduced with 0.05 ppm. The dose response curves for cytochrome P-450 content, NADPH-cytochrome P-450 reductase activity and 8-OHdG formation exhibited essentially the same patterns as for GST-P positive foci. A low dose of α-BHC also tended to up-regulate Ogg1 mRNA expression. Similar to the phenobarbital case, α-BHC treatment lead to increase in PCNA positive cells within the areas of GST-P positive foci at a dose of 500 ppm but decreased values at low doses. Though the response curves for CYP2B1 and 3A2 catalytic activity, protein levels and mRNA expression showed thresholds, CYP2C11 activity exhibited an inverted J-shape. This major constitutive male-specific isoform was thus found to be up-regulated by a low dose of α-BHC treatment at the transcriptional level and with regard to catalytic activity detected with 2α- and 16α-testosterone metabolites. Thus, CYP2C11 might take part in detoxification while CYP2B1 and 3A2 isoenzymes are considered to participate in bioactivation of α-BHC and increase its toxicity, given the correlation with GST-P positive foci and oxidative DNA damage. The non-linear threshold dose response observed at low doses with respect of CYP2B1 and 3A2 can be deemed a result of a multi-step process “turning on” orphan nuclear receptors, constitutive androstane receptors and the pregnane X receptor, which is known to regulate CYP2B1 and 3A2 transcription by binding as a heterodimer to the retinoid X receptor58,59. Furthermore, in the same study it was shown that glutathione-S transferase, which plays an important role in detoxifying α-BHC, demonstrates a threshold in its activity towards α-BHC at low doses57,60.

The possibility of a hormetic effect of α-BHC regarding formation of liver tumors in vivo, was further examined in F344 rats at doses from 0.01 to 500 ppm in the diet for 36 weeks after initiation of hepatocarcinogenesis with DEN (unpublished data). Incidences and multiplicities of liver tumors were found increased in a dose-dependent manner by α-BHC at doses of 0.5–500 ppm, while a tendency for decrease in their values was found in the low dose 0.01 and 0.1 ppm groups, similar to the case with rat liver preneoplastic lesions.

From these results it was concluded that α-BHC indeed exhibits hormesis regarding its hepatocarcinogenicity by mechanisms involving induction of detoxifying enzymes at low dose, as well as influencing free radical production and oxidative stress, and consequently pathological change in the liver. In these studies, the dose response relationship for GST-P positive foci was represented by a J-shape curve, in line with the previous investigation of this chemical using...
Possibility of a Hormesis for Hepatocarcinogenicity of DDT

Inhibitory effects on the induction of GST-P positive foci were also noted with low doses of another non-genotoxic carcinogen, DDT\textsuperscript{28}. First, in the study of Sukata et al., F344 rats, 21-day-old at the commencement, received DDT at doses from 0.005 to 500 ppm in the diet for 16 weeks. In another experiment Kushida et al.\textsuperscript{61} investigated the possibility of hormesis after DDT administration to F344 rats for 11 and 43 weeks following initiation of hepatocarcinogenesis with DEN. In both experiments the doses of 20 ppm and above were associated with dose-dependent induction of GST-P positive foci in the liver. In contrast, 0.005 and 0.01 ppm administration resulted in a tendency for decrease in values below the control level (Fig. 4). Histopathological analysis of liver nodules also revealed a tendency for decrease in the incidence and multiplicity of hepatocellular carcinomas in the low dose groups as compared to the DEN initiation controls. The multiplicity of total tumors also tended to decrease, although incidences were similar. Alteration of the GST-P positive foci in the low dose groups was correlated with a tendency for decrease in the CYP3A2 protein level as well as induction of IL-1 receptor type 1 (IL-1R1) and TNF-\(\alpha\) receptor type 1, whose ligands have roles in downregulating CYP3A2 and influencing cellular proliferation or apoptosis\textsuperscript{16}. IL-1R1 is known to be a cell surface molecule involved in cell signaling\textsuperscript{62}, while IL-1 inhibits regeneration of rat liver cells\textsuperscript{63} and tumor cell growth\textsuperscript{64}, and inhibitory actions of IL-\(1\beta\) on hepatocyte DNA synthesis are effected by iNOS gene expression and NO production under IL-1R1 control\textsuperscript{65}. It was found that within GST-P positive areas, cell proliferation was slightly lower in the 0.005 ppm DDT dose group than in the DEN only treated group\textsuperscript{16}. As observed in experiments with phenobarbital and \(\alpha\)-BHC, CYP2B1/2 and CYP3A2 protein levels in the liver microsomal fraction were significantly elevated by high doses of DDT. In line with previous results, 8-OHdG formation was significantly suppressed by a low dose of the chemical, presumably related to effective DNA repair and co-repair of endogenous damage, which may exceed formation of adducts\textsuperscript{61}. Oxidative stress in the low dose group was suggested to be decreased because of the lowered CYP3A2 expression and formation of 8-OHdG balanced through elimination by Ogg\textsuperscript{116,61}. Furthermore, in the low DDT dose group, mRNA expression and immunohistochemical staining of connexin 32 (Cx32) were found to be elevated\textsuperscript{16}. Many previous studies indicated that high doses of DDT and other non-genotoxic carcinogens inhibit Cx32, resulting in the loss of the function of gap junction intracellular communication (GJIC) and release of potentially initiated cells from growth constraints imposed by normal neighboring cells, resulting in clonal expansion and ultimately tumor formation and progression\textsuperscript{66–70}. In the present study, mRNA expression of one of the transcriptional factors, HNF-1\(\alpha\), which regulates Cx32 expression\textsuperscript{71,72}, was in good correlation with that of Cx32\textsuperscript{61}. Differential alteration of HNF-1\(\alpha\) is suggested to be one of the possible mechanisms by which DDT might inhibit or promote rat hepatocarcinogenesis.

Hormetic Effects Observed with Ethanol

Effects of alcohol intake on cardiovascular diseases\textsuperscript{73}, stroke\textsuperscript{74}, all causes of death\textsuperscript{73,75}, and cancer mortality\textsuperscript{76} are known to demonstrate U- or J-shaped curves; that is, those who consume a little alcohol have the lowest risk. The relationship between smoking or drinking dose and risk for stomach cancer has also attracted great interest as to whether strict dose-dependence or a U-shaped curve might be evident\textsuperscript{77}. Recently, the risk of stomach cancer was reported to increase linearly with the smoking dose, but not with the drinking dose. Kikuchi et al.\textsuperscript{78} showed that light drinkers in Japan have the lowest risk of developing stomach cancer among both male and female subjects, and heavy drinkers the highest risk among males, the association being J-shaped among male subjects, and U-shaped among female subjects, and thus very similar to the association with risk of cardiovascular diseases and stroke. J- or U-shaped dose-responses were suggested to offer an explanation for the fact that more studies on stomach cancer have demonstrated an association with smoking than with drinking\textsuperscript{78}.

In a recent study the promoting effects of ethanol at different doses on MeIQx induced liver carcinogenesis in
F344 rats was evaluated. No significant inhibitory activity on hepatocarcinogenesis was observed after administration of ethanol at low doses (0.1–1%), while a high dose of ethanol (10–20% in drinking water) was found to exert clear promotion of development of MeIQx induced liver cancer in rats.

Adaptive Mechanisms

To explain hormetic effects, adaptive responses have been proposed. When experimental animals are exposed to biologically effective levels of chemicals, their bodies have to deal with chemical perturbation and diverse responses are elicited. For some chemicals, the initial response constitutes an adaptive effect that maintains homeostasis. Disruption of this balance at any level of organization may lead to an adverse effect, or toxicity. When target cells are exposed to non-genotoxic carcinogens, as described above, it is to be expected that machinery to conserve homeostasis would be switched on, for detoxification and excretion, with preservation of the cell cycle and programmed cell death regulation through cell signaling. At very low doses of chemicals, such mechanisms in target cells might more than compensate for cell injury, so that not only a dose threshold but also a reduction in lesion development, as compared to the control case, may occur. This would explain the U- or J-shaped response curves obtained for phenobarbital, α-BHC and DDT hepatocarcinogenicity (Fig. 5).

Hepatic adaptive responses usually involve actions of the chemical on cellular signaling pathways, often receptor mediated, leading to changes in gene expression and ultimately alteration of the “metabolome”, directed toward maintaining homeostasis through modulation of various cellular and extracellular functions. At all levels of organization, adaptive responses are beneficial in that they enhance the capacity of all units to respond to chemical induced stress, are reversible and preserve viability. In contrast, adverse or toxic effects produced by genotoxic chemicals often involve chemical reactions with cellular macromolecules such as DNA or proteins and result in disruption of homeostasis. Such effects can be nonreversible at all levels of organization resulting in mutations or inactive protein molecules. Examples of compounds eliciting adaptive effects are provided by phenobarbital and ciprofibrate, whereas p-dichlorobenzene and 2-AAF, for instance, exhibit primarily toxic effects.

Hormetic Effects with Endogenous ROS

Exposure to different chemical carcinogens for which hormetic effects are proposed leads to formation of ROS, and frequently to induction of cytochrome P-450 species, with induction of oxidative stress. ROS are genotoxic in principle, and the question arises as to whether chemicals that increase ROS production will add to an endogenously produced background level of DNA lesions, or whether compensatory mechanisms exist that may result in non-linear dose-effects. Endogenous ROS cause detectable background levels of DNA damage, namely in the form of oxidized bases (e.g. 8-OHdG), apurinic (AP) sites, and strand breaks. Oxygen radicals also attack other cellular components such as lipids, to generate reactive intermediates that couple to DNA and give rise to exocyclic etheno- and propane-adducts and 1,N6-ethenodeoxyguanosine and 3,3′-ethenodeoxyctydine. Such adducts will have mutation-associated consequences upon cell replication. The continuous production of free radicals from radiation and other sources has stimulated organisms to evolve repair systems for oxidative base modifications or chromosome breaks. Alteration to DNA molecules triggers repair, and frequent activation may increase the general repair capacity, irrespective of the cause of the damage. Repeated exposure to ROS may thus lead to an adaptive response, mitigating the mutagenicity of oxidative DNA lesions. DNA repair is a crucial factor in maintaining a low steady-state level of DNA damage and its impairment is implicated in processes that promote human cancer. It is difficult to state at the present time the precise role of ROS-induced DNA damage in carcinogenesis and how genetic and epigenetic events induced by ROS interact with cell transformation and malignant progression. However, many aspects have already been elucidated, indicating that at low levels of ROS, adaptive responses, repair and antioxidative defenses are strengthened, whereas at high levels they may be overwhelmed. Whether induction of a detoxifying enzyme qualifies as a basis for a practical threshold depends on the speed and capacity of removal of the reactive species from the system compared with the speed of the translocation of the reactive species from the site of its generation to the nucleus and reaction with the DNA.

Bystander Effects

Numerous investigations have revealed that several cancer relevant effects of ionizing radiation can occur in cells that have received only cytoplasmic or plasmalemmal...
membrane exposure to ionizing radiation\textsuperscript{85–92}. Furthermore, many effects that have been attributed to ionizing radiation-induced damage to nuclear DNA or that occur following irradiation of the cytoplasmic compartment of cells can also occur in cells that have received no direct exposure to ionizing radiation. These so-called “bystander effects” as well as adaptive responses are linked to biological effects of radiation and chemical treatments and involve intracellular communication systems (both gap junctional and extracellular communication)\textsuperscript{85}. Bystander effects are considered to be induced by radiation in non-irradiated cells when an extracellular signal produced by a radiation-targeted cell is received by a non-hit cell, or by gap junctional direct transfer of some radiation-induced signals\textsuperscript{86}. Bystander effects may include increase in intracellular ROS, induction of mutations, enhanced cell growth, apoptosis, genomic instability and neoplastic transformation, as well as cell death\textsuperscript{87–92}. Both direct transfer of small molecules or ions through gap junctions and extracellular signaling by secreted factors (hormones, cytokines, growth regulators, etc.) maintain homeostasis and might be related to hormesis\textsuperscript{86}. The implications of bystander effects of low and high dose radiation exposure for potential health endpoints still need to be resolved.

**Dose Response in Cell Proliferation, Apoptosis and DNA Repair**

Induction of ROS has been observed to alter cell proliferation and apoptosis in the tissues. While marked increase in oxygen radicals in the rat liver in cases of non-genotoxic carcinogens phenobarbital, \(\alpha\)-BHC and DDT at high dose, for example, leads to elevation of PCNA indices in areas of GST-P positive foci, cell proliferation rates at low doses were found to be decreased\textsuperscript{14}. Suppression of liver nuclear DNA 8-OHdG formation at low dose may be associated with reduction of cell proliferation within GST-P positive foci. Furthermore, apoptosis, significantly induced by high dose administration in liver tissue surrounding GST-P positive foci, was suppressed in the low groups, with strong similarity to the pattern observed for 8-OHdG. Apoptosis of normal-appearing liver tissue has been proposed as one factor regulating the size of foci, as enlargement of GST-P positive foci presumably requires regenerative stimuli. In a low dose phenobarbital study, the results of CDNA microarray analysis indicated 2 ppm to specifically enhance mRNA expression for glutamic acid decarboxylase (GAD65), an enzyme involved in the synthesis of gamma-aminobutyric acid (GABA), while suppressing expression of MAP kinase p38, JNK1, 2 and other intracellular kinases\textsuperscript{14}. A negative correlation between the expression of GABA-A receptors in hepatocytes and thymidine incorporation in liver specimens was reported, albeit without evidence of a causal relationship, and the GABA-B receptor subtype is known to be involved in hepatocyte DNA synthesis and mediation of growth stimulation\textsuperscript{93,94}. Thus, the suppression of gene expression of signal transduction modulators, such as MAP kinase p38, JNK1, 2 and other intracellular kinases might be a factor related to the inhibitory effect of phenobarbital on cell proliferation.

The fact that DNA repair protects cells from fixation of DNA damage in the newly synthesized DNA strand as heritable mutations, means that outcome of exposure to carcinogens is dependent on the race between repair and proliferation-dependent DNA synthesis. The combination of elevated repair and decreased cell division may more than compensate for deleterious influence. Application of higher doses of the same substance may result in an increased tumor incidence because of cell cycle progression due to cytotoxicity and regenerative cell proliferation. As a consequence, a J-shaped dose-effect curve results. It is proposed that cell cycle progression and regenerative proliferation represent the key parameters concerning threshold mechanisms, although apoptosis also contributes. This would be particularly important for epigenetic carcinogens, whereas the genotoxic substance levels of DNA

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**Fig. 6.** Proposal of a flow scheme toward dose-effect relations, risk assessment and mechanisms of action of non-genotoxic chemical carcinogens.
damage in target tissues are far higher. Furthermore, it should be borne in mind that apoptosis and the control of neoplastically transformed cells by the immune system may be additional factors influencing the shape of the dose-effect curve.

Conclusions

In summary, recent data on the effects of non-genotoxic carcinogens, indicate the existence of hormesis and a “perfect” threshold for carcinogenicity (Fig. 6). Hormesis by non-genotoxic carcinogens implies the maintenance of homeostasis, with adaptive responses involving cell proliferation and apoptosis, DNA damage and repair, cell signaling, and cell-cell communication. The findings have broad implications for cancer risk assessment methods, experimental design, and the establishment of optimal drug doses, taking advantage of adaptive effects. Quantitative analyses based on biological models are necessary, with attention to factors that affect the degree of non-monotonicity. Further analyses along these lines should promote scientific discussion of biphasic dose responses and the concepts of “hormesis” and thresholds, particularly for tumor induction by non-genotoxic carcinogens.

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