REVIEW

Hormesis and dose–response-mediated mechanisms in carcinogenesis: evidence for a threshold in carcinogenicity of non-genotoxic carcinogens

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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 using phenobarbital, 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethane (DDT), α-benzene hexachloride (α-BHC) and other non-genotoxins. Furthermore, the effects of low and high doses of non-genotoxic and genotoxic compounds on carcinogenesis are compared, with especial attention to differences in mechanisms of action in animals and possible application of the dose–response concept to cancer risk assessment in humans. 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. Genotoxic agents, in contrast, cause genetic change by directly attacking DNA and inducing mutations, in addition to temporarily modulating the gene activity. Carcinogens can elicit a variety of changes via multiple genetic and epigenetic lesions, contributing to cellular carcinogenesis.

Chemical 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. On the other hand, errors in DNA replication themselves might cause mutations that are then inherited by progeny cells. 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 doses (1). Non-DNA-reactive compounds, such as topoisomerase inhibitors (2,3) and inhibitors of the spindle apparatus or associated motor proteins (4–7), are considered to act by this mechanism (8).

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 alteration (9). The indirect nature of the mechanisms involved means that prolonged exposure to high levels of chemicals is necessary for the production of tumors (10). 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, for example dioxin, do not bind directly to DNA but alter cell proliferation and physiology by inducing expression of enzymes involved in the 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 cells (11).

Threshold in carcinogenicity of environmental carcinogens

With the examination of the risk of human exposure to chemicals having carcinogenic potential, which are present 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 (8). The idea is that carcinogens can be further classified as follows: (i) genotoxic agents without a threshold in their effects; (ii) genotoxic compounds for which the existence of a threshold is possible but is

Abbreviations: α-BHC, α-benzene hexachloride; 2-AAF, 2-acetylaminofluorene; Cx32, connexin 32; DDT, 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT), α-benzene hexachloride (α-BHC) and other non-genotoxins. Furthermore, the effects of low and high doses of non-genotoxic and genotoxic compounds on carcinogenesis are compared, with especial attention to differences in mechanisms of action in animals and possible application of the dose–response concept to cancer risk assessment in humans. 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. Genotoxic agents, in contrast, cause genetic change by directly attacking DNA and inducing mutations, in addition to temporarily modulating the gene activity. Carcinogens can elicit a variety of changes via multiple genetic and epigenetic lesions, contributing to cellular carcinogenesis.

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not yet sufficiently supported; (iii) genotoxic carcinogens for
which a ‘practical’ threshold is supported by studies on mech-
anism(s) and/or toxicokinetics; (iv) genotoxic carcinogens for
which a ‘perfect’ threshold is associated with a non-observed
effect level (NOEL); and (v) non-genotoxic carcinogens for
which a ‘perfect’ threshold is associated with an NOEL (8).

Low-dose hepatocarcinogenicity of genotoxic environ-
mental carcinogens has been recently examined as an aid to
cancer risk assessment in humans and data pointing to ‘prac-
tical’ thresholds have been documented for 2-amino-3,8-
dimethylimidazo[4,5-f]quinoxaline (MeIQx), a food-derived
hepatocarcinogen, and diethylnitosamine (DEN). In the
MeIQx case, the carcinogen was administered to male F344
rats through the diet at various doses of 0.0001–100 p.p.m.
in 16 and 32 week studies (12). In a subsequent experiment it
was administered to rats for 4 weeks followed by 11 weeks of
phenobarbital treatment (13). In the DEN hepatocarcinogenic
study a total of 1957 F344 rats received the carcinogen at
doses of 0.0001–10 p.p.m. in their drinking water continuously
for 16 weeks (12). NOELs with regard to formation of gluta-
thonine S-transferase placental form (GST-P) positive foci, a
preneoplastic endpoint marker lesion for carcinogenesis in the
liver, were found to be 10 and 0.1 p.p.m. for MeIQx and DEN,
respectively (12). Data for GST-P positive focus development
with MeIQx followed by phenobarbital treatment at doses of
0.001–1 p.p.m. were similar to those with MeIQx-alone
(13). Coadministration of carbon tetrachloride (14) or ethanol
enhanced the induction of liver GST-P positive foci by MeIQx
in each group. MeIQx–DNA adduct formation in the liver
demonstrated a linear relationship with all the doses tested,
levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) being
linearly elevated, from 1 p.p.m. MeIQx at week 4 and from
0.01 p.p.m. MeIQx at week 16. Interestingly, in a Big Blue
transgenic rat mutagenesis assay, MeIQx at doses of ≤1 p.p.m.
was found not to induce lacI gene mutations in the liver. This
closely correlates with non-induction of GST-P positive foci
(15). However, the dose of MeIQx at which in vivo muta-
genicity was significant, was lower than that for induction of
GST-P positive foci. Increase of carcinogen-DNA adducts,
8-OHdG, in vivo mutagenicity, induction of GST-P positive foci
and lastly liver tumors appeared to be the chain of sequen-
tial events dependent on the dose of carcinogen, indicating
the existence of NOELs and implying at least a ‘practical’
threshold for carcinogenicity of genotoxic carcinogens such
as MeIQx and DEN. Data in line with these results were
also obtained in low dose studies of 2-amino-1-methyl-6-
phenylimidazo[4,5-b]pyridine carcinogenicity in the rat
colon (16).

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 (17) and 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 poten-
tial 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 the substances acting via epigen-
etic mechanisms, such as phenobarbital (18,19), α-benzene
hexachloride (α-BHC) (20), 1,1-bis(p-chlorophenyl)-2,2,2-
trichloroethane (DDT) (21), 2,3,7,8-tetrachlorodibenzo-p-
dioxin (TCDD) and caffeine (22). However, genotoxic
carcinogens, such as 2-acetylaminofluorene (2-AAF) (23,24)
and ionizing radiation (IR) (25), may also be included. Inhibi-
tory effects of all these agents at low doses on carcinogenesis
have been subsumed under the heading of hormesis (17).

The theory of hormesis

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 (26). Hermetic effects have
been studied for more than two decades (27) and many toxici-
cants have shown benefits, rather than harm, with low-level
exposure.

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 (26). This provided a toxicological explanation for his
development of homeopathic ideas. As a result of the publicity
following these initial studies he became the main academic
hero for numerous advocates of homeopathy, and thus the
theory of hormesis was born in close association with homeo-
pathy as a preventive/therapeutic modality (26). 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, USA and Japan (26). 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 (28). Hormesis implies
the existence of a threshold dose level and there are dose–
response models that include parameters that account for the
threshold.

With IR, hormesis was interpreted to be due to adaptation to
background radiation exposure, as well as metabolic protec-
tion against the array of other abiotic stresses in the environ-
ment (25,29). 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 trans-
formation in non-irradiated cells (30). 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. 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 (30).

Hormesis in carcinogenesis

The question whether the concept of hormesis can be
generalized to carcinogenesis has been recently discussed by
E. Calabrese and L.A. Baldwin (31, 32). They cite numerous examples in well-designed studies providing evidence for U- and J-shape dose relationships with respect to different biomarkers of carcinogenesis in different animal models. For some chemicals tested, carcinogens were found to be similar to other toxicants in improving the outcome at low doses, although the mechanisms of their action remained unclear. Therefore, it appears very important to answer the question of how carcinogens act at very low doses. Early stage carcinogenesis includes initiation with the occurrence of DNA damage and adaptative DNA repair. In 1983, Camurri et al. (33) observed a decrease of chromosomal aberrations with low-dose styrene treatment. The response of human keratinocytes to a low dose of the well-known methylating agent, N-methyl-N’-nitro-N-nitrosoguanidine, was studied by Kleczkowska and Althaus (34). It was found that at concentrations in the 0.05–50 nM range DNA unwinding and DNA strand breaks were significantly reduced, while at high doses they were enhanced compared with the control case. Inhibition activity regarding DNA damage at low doses was explained by activation of poly(ADP)-ribose. Furthermore, assessment of the effects of Hg$^{2+}$ on $O^6$-methylguanine-DNA methyltransferase activity of human buccal fibroblasts by Liu et al. (35) revealed elevation at low doses of 0.3–3 μM. In the dose–response curves of rat hepatic DNA damage for different types of carcinogens assessed by Kitchen and Brown (36), 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 caffeeic acid in the rat forestomach and kidney, altered hepatic foci formation with TCDD in DEN-pretreated partially hepatotichomentized rats (22) and urinary bladder hyperplasia in saccharin-treated rats (37). 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 (38). In addition, TCDD at hepatocarcinogenic doses was reported to be capable of causing dose-dependent reduction in mammary and uterine tumors (39). In 1994, Cook (40) 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 (25) or metals such as selenium (41). U-shape responses were also observed for chemically induced pulmonary tumors (42–44) and testicular cancer (45).

### Hormesis in phenobarbital hepatocarcinogenicity

Recently, especial attention has been devoted to the carcinogenicity of low doses of phenobarbital, a sedative and anti-convulsant, 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 cancers in humans, indicating that humans may have low sensitivity to toxic effects of phenobarbital. In the rat, Goldsworthy, et al. (46) reported no promotion by phenobarbital <10 p.p.m. with regard to the enzyme-altered foci. Furthermore, Kitagawa (47) 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. (48) found a slight decrease of altered hepatic foci by 10 p.p.m. phenobarbital and Maekawa et al. (49) demonstrated similar results with 1 p.p.m. phenobarbital. To determine the practical threshold level for hepato-promoting effects of phenobarbital, Kitano et al. (18) investigated dose dependence using a rat liver medium-term bioassay (Ito test) (50).

When phenobarbital was administered to rats in a wide range of doses of 0.01–500 p.p.m. in the diet for 6 weeks after a single intraperitoneal injection of DEN in serial experiments, GST-P positive foci were found to be increased dose dependently in rats that were given 60–500 p.p.m. However, with doses in the range of 1–7.5 p.p.m., decrease was evident as compared with the control group, this being statistically significant at 1 and 2 p.p.m. (Figure 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 the suppression of cytochrome P-450 CYP3A2 protein expression by low doses of the chemical (18).

For further clarification of the hormetic influence of phenobarbital, Kinoshita et al. (19) investigated doses of 0, 2, 15 and 500 p.p.m. applied in diet to male F344 rats for 10 or 33 weeks after initiation of hepatocarcinogenesis using DEN. Formation of GST-P positive foci and liver tumors was inhibited at 2 p.p.m. after 10 and 33 weeks of phenobarbital administration, respectively (Figure 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 p.p.m. (19). In contrast, a 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, and 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 oxoguanine glycosylase 1 (Ogg1), which is responsible for the repair of 8-OHdG lesions. 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 regeneration (19). Another explanation for the suppressive effect of phenobarbital on the development of preneoplastic lesions might involve stimulation of hepatic drug-metabolizing enzymes, which detoxify carcinogens (48). Activation of P-450 isoenzymes CYP2C11 and NADPH-cytochrome P-450 reductase (OR) in liver microsomes observed after the administration of phenobarbital at a low dose, if not accompanied by elevation of their protein expression leading to the generation of large amount of OH, might have a protective effect (19). The available results thus indicate that the compound exhibits hormetic effects on rat hepatocarcinogenesis initiated using DEN by differentially altering cell proliferation, apoptosis and oxidative DNA damage at high and low doses.
**Fig. 1.** Induction of GST-P positive foci in the livers of rats treated with phenobarbital in a medium-term bioassay (Ito test). PH, 2/3 partial hepatectomy.

**Fig. 2.** Hepatocarcinogenicity of phenobarbital in the rat liver: GST-P positive foci and tumor development (DEN→PB). See online Supplementary material for a color version of this figure.
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. Among the eight isomers of BHC, the α-isomer has been categorized as a non-genotoxic carcinogen as it induces liver tumors in rodents after high-dose administration in the long-term, but no mutagenicity is shown in the Ames test. The major metabolite in α-BHC metabolism by the cytochrome P-450 oxidoreductase system is 2,4,6-trichlorophenol. 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-deoxyuridine and 8-chloro-2-deoxyadenosine (51,52). Long-term treatment with high doses of α-BHC (such as 500 or 1000 p.p.m.), but not β- and γ-BHC, has been found to induce hyperplastic nodules and carcinomas in the livers of rats and mice (53,54). Early toxicological studies revealed that α-, β- and γ-BHC are potent inducers of hepatic monoxygenases in rats (55), in addition to causing liver enlargement (56,57). Since induction of the monoxygenase system is assumed to influence the promotion stage (58,59), the mechanism of α-BHC carcinogenicity is likely to be due to its influence on spontaneously initiated hepatocytes (53,54).

To investigate whether α-BHC exhibits hormesis with respect to its hepatocarcinogenicity the dose dependence of its promoting effects was first investigated by Masuda et al. (20) 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 p.p.m. 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 0.5–500 p.p.m. However, a tendency for a decrease was observed with 0.01 and 0.1 p.p.m. α-BHC (Figure 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 of rat hepatocarcinogenesis by α-BHC and suggested that the mechanism might be related to the suppression of P-450 isoenzyme CYP3A2 protein expression by low doses (20).

A second study was conducted with α-BHC applied to F344 rats at doses of 0.01–500 p.p.m. for 10 weeks after DEN initiation (unpublished data). While α-BHC promoted the formation of GST-P positive foci at the dose of 500 p.p.m., both the numbers and areas of preneoplastic lesions were found to be significantly reduced with 0.05 p.p.m. 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 upregulate Ogg1 mRNA expression. Similar to the phenobarbital case, α-BHC treatment led to increase in PCNA positive cells within the areas of GST-P positive foci at a dose of 500 p.p.m. but gave 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 isozyme was thus found to be upregulated 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 to be 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 receptor, RXR (60,61). 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 doses (62, unpublished data).

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 p.p.m. given in the diet for 36 weeks after initiation of hepatocarcinogenesis using 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 p.p.m., while a tendency for decrease in their values was found in the low-dose 0.01 and 0.1 p.p.m. groups, similar to the case with rat liver preneoplastic lesions (unpublished data).

From these results it was concluded that α-BHC exhibits hormesis with regard to its hepatocarcinogenicity at low dose by mechanisms involving induction of detoxifying enzymes, as well as by influencing free radical production and oxidative stress, and consequently bringing pathological change in the liver. In these studies, the dose–response relationship for GST-P positive foci was represented using a J-shape curve, in line with the previous investigation of this chemical using the Ito test (20).

**Possibility of a hormesis for DDT in hepatocarcinogenesis**

Inhibitory effects on the induction of GST-P positive foci were also noted with low doses of another non-genotoxic carcinogen, DDT (21). First, in the study of Sukata et al. (21), F344 rats, 21-day-old at the commencement, were administered DDT at doses from 0.005 to 500 p.p.m. in their diet for 16 weeks. In another experiment Kushida et al. (63) investigated the possibility of hormesis after DDT administration to F344 rats for 11 and 43 weeks following initiation of hepatocarcinogenesis using DEN. In both experiments the doses of ≥20 p.p.m. were associated with dose-dependent induction of GST-P positive foci in the liver. In contrast, 0.005 and 0.01 p.p.m. administration resulted in a tendency for decrease in values below the control level (Figure 4). Histopathological analysis of liver nodules also revealed a tendency for decrease in the incidence and multiplicity of HCCs in the low-dose groups as compared with 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 I (IL-1RI) and TNF-α receptor type I, whose ligands have roles in downregulating CYP3A2 and influencing cellular proliferation or apoptosis (21). IL-1RI is known to be a cell surface molecule involved in cell signaling (64), while IL-1 inhibits regeneration of rat liver cells (65) and tumor cell growth (66), and inhibitory actions of IL-1β on hepatocyte DNA synthesis are effected by iNOS gene expression and NO production under IL-1RI control (67).

It was found that within GST-P positive areas, cell proliferation was slightly lower in the 0.005 p.p.m. DDT dose group than in the only DEN treated group (21). As observed in experiments with phenobarbital and α-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 (68). 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 Ogg1 (21,63). Furthermore, in the low DDT dose group, mRNA expression and immunohistochemical staining of connexin 32 (Cx32) were found to be elevated (21). 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) (60–71) and release of potentially initiated cells from growth constraints imposed by normal neighboring cells, resulting in clonal expansion and ultimately tumor formation and progression (71,72). In the present study, mRNA expression of one of the transcriptional factors, HNF-1α, which regulates Cx32 expression (73,74), was in good correlation with that of Cx32 (62). Differential alteration of HNF-1α 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 (75), stroke (76), all causes of death (75,77) and cancer mortality (78) are known to demonstrate U- or J-shaped curves; that is, those who consume very less alcohol have the lowest risk. The relationship between smoking dose 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 (79). Recently, the risk of stomach cancer was reported to increase linearly with the smoking dose, but not with the drinking dose. Kikuchi et al. (80) 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–response curves were suggested to offer an explanation for the fact that more studies on stomach cancer have demonstrated an association with smoking than with drinking (80).

In a recent study the promoting effects of ethanol at different doses on MeIQx induced liver carcinogenesis in F344 rats was evaluated (unpublished data). 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, no significant inhibitory activity on hepatocarcinogenesis was observed after the administration of ethanol at low doses (0.1–1%).

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 (24,26). 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 mechanisms in target cells might more than compensate for 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 non-reversible 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.

Bystander effects
Numerous investigations have revealed that several cancer relevant effects of IR can occur in cells that have received only cytoplasmic or plasmalemmal membrane exposure to IR (81–88). Furthermore, many effects that have been attributed to IR-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 IR. 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) (81).

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,N4-ethenodeoxycytidine (89–91). Such adducts will have mutation-associated consequences upon cell replication (92). 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

Fig. 5. Potential mechanisms mediating hormesis in carcinogenesis.
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 (93). 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 or not the 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.

Alteration to 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, for example phenobarbital, α-BHC and DDT at high dose, leads to elevation of PCNA indices in areas of GST-P positive foci. Cell proliferation rates at low doses were found to be

Fig. 6. Proposal of a flow scheme toward dose–effect relations, risk assessment and mechanisms of action of non-genotoxic chemical carcinogens.
decreased (19). 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 foci was suppressed in the low dose 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 p.p.m. of phenobarbital 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 (19). Recently, 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 (94,95). Thus, the suppression of gene expression of signal transduction modulators, such as MAP kinase p38, JNK1 and 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 to this. This would be particularly important for epigenetic carcinogens, whereas the genotoxic substance levels of DNA damage in target tissues are far higher. Of high interest are genotoxic substances like MeIQx or DEN for which carcinogenicity, induction of regenerative proliferation and genotoxicity also appear to act through processes with a threshold. 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 (Figure 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–response curves and the concepts of ‘hormesis’ and thresholds, particularly for tumor induction by non-genotoxic carcinogens.

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References


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