Threshold in Carcinogenicity of Genotoxic Carcinogens

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Abstract

Nowadays the idea of threshold in the carcinogenicity of chemical carcinogens has attracted interest in the field of carcinogenesis. With genotoxic agents there is considerable experimental evidence in support of the idea. Here, we report on the low dose carcinogeticity in rats observed with heterocyclic amines contained in cooked food, 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MelQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), and contaminants of natural and manufactured food products, N-nitrosocompounds such as N-nitrosodimethylamine (DEN) and N-nitrosodimethylamine (DMN). The existence of a no-effect level for MelQx carcinogeticity was confirmed in a medium-term rat liver bioassay. Treatment with increasing doses of MelQx caused sequence of events to occur in the liver tissue: first, the induction of DNA-MelQx adducts at low doses, then an increase of DNA 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation and lacI gene mutations, following by the development of preneoplastic lesions, glutathione S-transferase placental form positive (GST-P+) foci, at high doses. In another study, IQ was found to induce preneoplastic lesions in the rat liver at high doses, but lack any effect at low doses. Similarly, examination of carcinogenicity of a well-known colon genotoxic carcinogen PhIP have shown that application at low doses caused the formation of PhIP-DNA adducts, however, a surrogate marker of preneoplastic lesions in the colon, aberrant crypt foci, were detected only at high doses. In studies with N-nitrosocompounds, no GST-P+ foci in the rat livers was detected after the treatment at low doses, on the contrary, at high doses DEN and DMN induced their development. In conclusion, DNA-reactive genotoxic agents such as heterocyclic amines MelQx, IQ and PhIP, and N-nitrosocompounds DEN and DMN were concluded to exert a threshold, at least practical, with respect to their carcinogenicity.

Keywords: Genotoxic carcinogens; Carcinogenic threshold; Heterocyclic amines; N-nitrosocompounds

Introduction

Chemical carcinogens are divided into two classes, genotoxic and non-genotoxic, on the basis of their ability to react with nuclear DNA and form adducts. In most studies, their effects are experimentally examined when carcinogens are administered at high doses, including the maximum tolerated dose. In the cancer risk assessment, it has been considered that the dose response curve for carcinogenicities of non-genotoxic carcinogens shows a no-response level at low dose, indicating the existence of carcinogenic threshold. However, in case of genotoxic carcinogens, the curve has been always thought to reach zero, indicating that even at low doses there might be a carcinogenic effect. The "no-threshold concept" in the field of risk assessment for genotoxic carcinogens is based on this statement which means the absence of threshold in carcinogenic potential means that even at very low doses genotoxic carcinogens could have an influence on humans. Nevertheless, this concept appears to be a putative theory, since it is not proved experimentally whether those carcinogens are able to exert carcinogenicity at low doses. Whether it is appropriate or not to extrapolate the effects of exposure at high doses to low doses is still a question of interest. Therefore, it is very important to resolve this concept from the viewpoint of cancer risk assessment and management.

For some chemicals, the initial response constitutes an adaptive effect that maintains homeostasis [1,2]. Disruption of this balance at any level of organization may lead to an adverse effect or toxicity. Adverse or toxic effects produced by genotoxic chemicals often involve chemical reactions with cellular macromolecules (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. Recently biological adaptive responses, resulting in physiological protection have become recognized in radiation carcinogenesis [3]. This concept might be also useful for understanding dose effects in chemical carcinogenesis, since adaptation might be expected in response to low doses of all DNA-damaging agents. Adaptive responses usually involve actions of the chemical on cellular signaling pathways, often receptor mediated, leading to changes in gene expression and metabolism, stimulation of immune response, induction of detoxification and repair systems enzymes and upregulation of tumor suppressor genes. At all levels of organization, adaptive responses are beneficial because they enhance the capacity of organism to respond to chemically induced stress, reversible and preserve viability.

DNA-reactive genotoxic carcinogens, which are mostly mutagenic, are metabolized to ultimate carcinogens and to bind with DNA in target organ cells forming DNA adducts, inducing gene alterations, and exerting carcinogenicity. DNA damage induced by carcinogen is efficiently repaired, however, if the DNA repair errors occur, some of adducts give rise to miss repair, resulting in fixation of mutations and appearance of mutated cells [4]. Apoptosis as well as the DNA repair helps to maintain the normal condition of the tissues and organs. It has been proposed that these events occur during the initiation stage of chemical carcinogenesis. In addition, elevation of cell proliferation and evading apoptosis influences the preneoplastic lesions to develop...
quickly from mutated cells and to give rise to neoplasms, which are developed in the promotion and progression stages. It is important to mention, that evaluation of DNA adduct levels has become a very good biomarker for exposure assessment [5]. Those included heterocyclic amines (HCAs) MeIQx, IQ and PhIP DNA adducts as well as oxidative DNA damage marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) [6-12].

HCAs are formed during the cooking of meats by condensation of creatinine with amino acids. In recent studies with MeIQx, IQ and PhIP, their content in food was shown to be influenced by numerous factors including temperature, duration and type of thermal processing (grilling, fying etc.), material (beef, pork, poultry, fish, etc.), content of the substrates participating in their synthesis (sugars and amino acids), spices, natural and synthetic antioxidants, pH and duration of storage of fresh materials prior to processing [13]. In general, the dietary intake of these three HCAs is greatest for PhIP, followed by MelQx and IQ. It was also reported that considerably more PhIP is formed in poultry, while more MelQx is formed in beef, pork and fish [14]. It has been further demonstrated that the heterocyclic aromatic amines in food exist in free and bonded states (bounded to natural polymers, such as proteins, DNA and glycogen) [15]. In the model of the human digestive tract, HCAs are gradually released from their physical and chemical bonds during in vitro digestion of meat. The increase of HCAs content during the digestive process was suggested to be the result of their release under the influence of digestive enzymes like pepsin, endopeptidases trypsin, chymotrypsin and elastase and exopeptidases, which is likely to be catalysed by the temperature and different chemical composition of meat, for example the presence of iron Fe2+ and copper Cu2+ [15].

In this review we survey the examples and report on the carcinogenicities of genotoxic carcinogens at low and high doses which were examined in vivo studies using animal carcinogenesis models from the view point of the carcinogenic mechanism.

**Hepatocarcinogenicity and Mutagenicity of 2-Amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx)**

MeIQx is one of the most abundant carcinogenic HCAs in cooked foods, speculated to be a human liver carcinogen. MeIQx at doses of 100 to 400 ppm was found to be carcinogenic in the rat liver [7]. It is considered to exert genotoxic activity after metabolic activation by cytochrome P450 isoenzyme CYP1A2 and then N-acetyltransferase (NAT) [16]. Recent studies have shown that MeIQx induces expression of genes encoding metabolic enzymes CYP1A1, CYP1A2 and uridine diphosphate-5′-glucuronosyltransferase type 1A (UGT1A1), and expression of p53 and its downstream regulated genes cyclin dependent kinase inhibitor 1 (CDKN1A), also known as p21waf1/cip1, growth arrest and DNA damage-inducible 45 alpha (GADD45a) and apoptosis-related protein BAX [16].

A summary of recent key in vivo findings obtained in experiments with several genotoxic carcinogens is presented in Table 1. The effect of MeIQx exposure at different doses was investigated in 1145, 21-day-old male F344 rats. The chemical was administered in the diet at doses of 0, 0.001, 0.01, 0.1, 10 ppm (low dose groups) or 100 ppm (high dose group) for 16 and 32 weeks [17,18]. The lowest dose 0.001 ppm was established as equivalent to the daily intake of this carcinogen in humans (0.2 to 2.6 mg/kg/day). In a 16-week experiment, the total numbers and areas of rat liver glutathione S-transferase placental form positive (GST-P) foci, which are preneoplastic lesions and the end point marker in rat hepatocarcinogenesis, were not changed in the 0.001-1 ppm MeIQx groups, but, at 10 ppm and 100 ppm a trend for an increase and a significant elevation were observed, respectively, as compared to non-treated controls (Figure 1A and Table 1). Furthermore, in 32-week study, numbers of GST-P+ foci were significantly increased after the treatment with MeIQx at 10 ppm, and particularly at 100 ppm as compared to respective controls (Figure 1A). In addition, the formation of MeIQx-DNA adducts was dose-dependently induced after 4 and 16 weeks of carcinogen application (Figure 1B and data not shown, Table 1). Moreover, administration of MeIQx at 1 ppm and higher doses for 4 and 16 weeks caused significant elevation of 8-OHdG levels in nuclear DNA (Figure 1C and data not shown, Table 1).

Similarly, the mutation level of H-ras gene, which role in rat hepatocarcinogenesis is not clear, was significantly increased in the livers of rats administered MeIQx at doses higher than 1 ppm (Table 1) [19]. After the 1-week application of MeIQx in the diet at wide range of doses, H-ras mutation frequency detected by TCEL assay in the rat livers of all groups receiving from 0.001 ppm to 100 ppm dose of MeIQx did not differ from the control value. On the other hand, in the livers of rats treated for 2 weeks, H-ras mutation frequency was elevated in dose-dependent manner, particularly in 10 ppm and 100 ppm MeIQx groups with statistical significance [19].

Next, the mutagenicity of MeIQx in terms of the mutation level of the lacI gene in livers of Big Blue rats with genetic background of F344 was examined [20]. Significant elevation and a marked increase of lacI gene mutation levels was detected after 16 weeks of treatment with MeIQx at a dose of 10 ppm and 100 ppm, respectively (Figure 1D and Table 1). From these results, the existence of a no-effect level for the mutagenicity of MeIQx has been demonstrated. Importantly, in MeIQx-treated rats, the formation of GST-P+ foci was significantly induced only at a dose of 100 ppm in line with our previous results (data not shown).

When the initiation activity of MeIQx was examined in a 2-stage carcinogenesis model using 850, 21-day-old male F344 rats with phenobarbital as promoter of hepatocarcinogenesis, GST-P+ foci in the rat livers were significantly increased in the 10 and 100 ppm dose groups, while no difference was found at doses of 1 ppm or less compared to non-treated controls (data not shown) [18,21].

Susceptibility to hepatocarcinogenesis varies considerably among different strains of rats. Strain differences may exist in dose-response curve for MeIQx carcinogenicity. For clarification, we compared the effects of low dose MeIQx administration on hepatocarcinogenicity induced BN and F344 rats. Similar results were observed with both rats strains. Low doses (10 ppm and less) of MeIQx had no effect on induction of GST-P+ foci, although they were significantly increased at high doses (data not shown) [22].

Next, for the evaluation of the hepatocarcinogenicity of concurrent treatment of MeIQx and a typical genotoxic hepatocarcinogen, diethylnitrosamine (DEN), two 16-week rat hepatocarcinogenesis assays were further performed using a total of 790 male F344 rats [23]. In the first study, the effects of concurrent treatment of a subcarcinogenic dose of DEN on rat hepatocarcinogenesis induced by various doses of MeIQx were evaluated. In the second, the hepatocarcinogenicities of combinations of MeIQx and DEN at subcarcinogenic, low carcinogenic and high carcinogenic doses were examined. The concurrent treatment with subcarcinogenic doses of DEN did not enhance MeIQx-induced hepatocarcinogenicity which was evaluated in terms of GST-P+ foci development. Furthermore, concurrent treatment with combinations of subcarcinogenic doses of DEN and MeIQx was not hepatocarcinogenic, indicating that the combined effects of subcarcinogenic doses of DEN and MeIQx were neither additive nor synergistic. In addition,
concurrent treatment with low carcinogenic doses of these 2 carcinogens did not show additive or synergistic effects. However, in case of co-administration of MeIQx and DEN at high carcinogenic doses synergetic effects were found. Thus, the existence of no-effect levels of combinations of MeIQx and DEN was demonstrated, which provided new evidence for the idea of existence of a threshold for the carcinogenicities of genotoxic carcinogens.

The carcinogenic potential of MeIQx at low doses and the human
Carcinogenicity of 2-amino-3-methylimidazo[4,5-b]pyridine (PhIP) in the rat colon

A heterocyclic amine, PhIP, has attracted particular attention as a potential human colon genotoxic carcinogen, as humans are in fact exposed to continuous low doses of HCAs during lifetime. PhIP was shown to be metabolized by CYP1A2 [29], and to exert carcinogenicity in the rat colon [30]. Furthermore, recent data indicated that PhIP could cause stomach injury, oxidative stress in rat stomach as well as the activation of c-fos and c-jun and inactivation of p16, which may play a role in the pathogenesis of PhIP-associated stomach cancer [31]. Moreover, PhIP induced signal transducer and activator of transcription 1 (Stat1) and vascular endothelial growth factor (VEGF) expression which was suggested to be involved in PhIP-enhanced colon tumorigenesis in the post-initiation phase [32] (Table 1) (Figure 3). Furthermore, recent studies reported that down-regulation of breast cancer resistance protein (BCRP) expression in murine colon adenomas leads to an accumulation of PhIP in the above-mentioned lesions [33], thus suggesting that BCRP is an important PhIP efflux transporter.

To investigate carcinogenicity of PhIP in the rat colon, 1920, 6-week-old F344 male rats were administered carcinogen at a range of doses from 0.001 (human exposure level) to 400 ppm in their diet for 16 weeks [34]. The development of aberrant crypt foci (ACF), which are considered as a surrogate marker of the preneoplastic lesions in the colon [35], was not altered by PhIP application at doses of 0.001 to 10 ppm, however, at the doses of 50 to 400 ppm a significant increase in their number was observed (Figure 4 and Table 1). Furthermore, significant elevation of PhIP-DNA adduct level was detected in the groups treated with doses of 0.01 ppm and higher.

Next, we focused on the carcinogenicity of PhIP in the rat large intestine at various dose levels in initiation and promotion models of carcinogenesis. A total of 1926-week-old male F344 rats were subcutaneously injected twice with 15 mg/kg body weight azoxymethane (AOM), then continuously fed various doses (0, 0.001, 0.01, 0.1, 1, 10 and 100 ppm) in the diet for 16 weeks. Treatment with doses of 1 ppm and below did not induce GST-P+ foci in the liver, while 10 and 100 ppm doses application resulted in their development. Thus, the presence of no-effect levels of IQ for the liver carcinogenicity in the rat was demonstrated. The mechanism was proposed to be related to significant up-regulation of p21WAF1/Cip1 by IQ at doses below those required for its mediated carcinogenic effect in the liver. It has been suggested that suppression of cell cycle progression by p21WAF1/Cip1 followed by DNA repair is at least one of the mechanisms responsible for the observed no effect of low doses of IQ in rats in this model. Furthermore, IQ administration at doses of 0.01-10 ppm induced elevation of IQ metabolizing enzyme CYP1A2, while 100 ppm IQ caused up-regulation of CYP1A1 rather than CYP1A2. Significant induction of APE-1 and GADD45 was observed only at the highest doses of 10 and or 100 ppm, thus indicating that the IQ-induced DNA damage response is dose-dependent (Figures 2A and 2B).

Table 1: Key in vivo findings observed with several genotoxic carcinogens.

<table>
<thead>
<tr>
<th>Chemical Key</th>
<th>in vivo findings</th>
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<tr>
<td>MeIQx</td>
<td>Dose-dependent increase of MeIQx-DNA adducts, elevation of 8-OHHG, HAC, and H-ras mutation levels, and induction of GST-P+ foci and liver tumors (100 to 400 ppm) in rats. Existence of a no-effect level (1 ppm) for the carcinogenicity of MeIQx in the rat liver (4, 16, 32-week assays in F344, BN and BigBlue rats, 2-step carcinogenicity test, 2-year carcinogenicity test, medium-term rat liver bioassay). Existence of low-effect levels of combinations of MeIQx and DEN treatments in the rat liver (16 weeks assays of concurrent treatment with DEN and MeIQx). Metabolic activation by CYP1A2 and NAT. Induction of CYP1A1, CYP1A2, UGT1A1, P53, p21WAF1/Cip1, GADD45x and BAX.</td>
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<tr>
<td>IQ</td>
<td>Existence of no-effect level (0.001 ppm) for the carcinogenicity in the rat liver (16 weeks assay). Induction of CYP1A1, CYP1A2, APE1, GADD45x, GSTp1WAF1/Cip1 (10 ppm and below)</td>
</tr>
<tr>
<td>PhIP</td>
<td>Existence of no-effect level (10 ppm) for the carcinogenicity in the rat colon (16 weeks assay). Induction of oxidative stress, Stat1, VEGF, c-fos, c-jun, inactivation of p16 and BCRP.</td>
</tr>
<tr>
<td>DEN</td>
<td>Existence of no-effect level (0.01 ppm) for the carcinogenicity in the rat liver (16 weeks assay).</td>
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<tr>
<td>DMN</td>
<td>Existence of no-effect level (0.1 ppm) for the carcinogenicity in the rat liver (16 weeks assay).</td>
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0.01, 0.1, 1, 10, 50 and 200 ppm) of PhIP in the diet up to 16 and 36 weeks for the analysis of ACF and colon tumors (36). PhIP was found to enhance strongly AOM-initiated rat large intestinal tumorigenesis at high doses (50 and 200 ppm), while lower doses (0.001-10 ppm) had no apparent effects. High doses also caused variation in tumor histological types and their distribution throughout the large intestinal segments. Frequencies of ACF/cm² did not meaningfully vary between the groups. Cellular proliferation activity in normal-appearing colonic mucosa was significantly increased at high doses. These findings provided new evidence of a low-dose potential for PhIP, with a no-effect level to be 10 ppm in this initiation-promotion experimental model [36].

Hepatocarcinogenicity of N-nitrosocompounds in rats

Typical rodent hepatocarcinogens, N-nitrosocompounds (e.g. DEN and dimethylnitrosamine (DMN)) which mode of action is still unclear, have been shown to be synthesized in the stomach through the reaction of secondary amines and nitrites. Furthermore, they are contained in different life-substances and known as contaminants of different natural and manufactured food products. Metabolic oxidation of dialkylnitrosamines, during which they are activated and transformed into direct-acting mutagens, is known to carry out by two systems: one utilizes porphyrin and oxidant as a model for shunt pathway in the metabolizing pathway of cytochrome P450, and the other one utilizes Fenton reagent [37].

The carcinogenicity of DEN with respect to the relationship between the applied dose and reactivity was investigated by Peto and colleagues using 4080 male rats [38]. DEN at doses of 0.033 to 13.896 ppm was administered to rats in their drinking water, and induction of liver cancer was found to be dependent on the applied dose of DEN. Therefore, from the existence of the relationship between the treatment dose and tumorigenicity, it was concluded that DEN had no threshold for its carcinogenicity in the rat liver. However, as it has been noticed that experimentally examined low dose levels are still high as compared to the levels of human exposure, for clarification of the existence of the threshold in DEN hepatocarcinogenicity, we have decided to examine the influence of DEN applied at lower doses than those used by Peto et al. [38]. The doses of DEN and DMN were established in animal studies with reference to the human daily exposure which is about 0.0001 ppm [39,40].

Thus, in our next study, to investigate the carcinogenicity of low doses of DEN in the rat liver, 2000, 21-day-old male F344 rats were administered DEN over a range of doses from 0.0001 to 1 ppm in their drinking water for 16 weeks (Table 1 and Figure 4) [17]. No induction of GST-P+ foci was observed at DEN doses of 0 to 0.01 ppm, however, their values were significantly elevated at doses of 0.1 ppm or higher, demonstrating the no-effect level for DEN hepatocarcinogenicity.

When the hepatocarcinogenicity of another N-nitrosocompound, DMN, was examined using the same experimental assay, in which the carcinogen was applied to F344 rats at doses of 0.0001 to 10 ppm in their drinking water for 16 weeks, no induction of GST-P+ foci was found at doses of 0.001 to 0.1 ppm, but the significant increase of their numbers and areas was observed at 1 and 10 ppm (Table 1 and Figure 4) [41]. It was concluded that similarly to DEN, DMN has no-effect level for its hepatocarcinogenicity in rats.

Conclusion

Our recent data on the effects of DNA reactive genotoxic carcinogens such as HCAs, MeIQx, IQ and PhIP, and N-nitrosocompounds, DEN...
and DMN, in animal models indicate the existence of threshold, at least practical, with respect to their carcinogenicity, which implies the maintenance of homeostasis, with adaptive responses involving alteration to formation of DNA damage, DNA adducts, gene alteration and repair, apoptosis, cell proliferation and cell signaling. This conclusion is very important regarding how we should view the impact of genotoxic carcinogens in human environment in relation to cancer risk assessment and management. Genotoxic carcinogens (e.g. MeIQx), are suggested first to induce formation of DNA adducts at low doses, then elevation of 8-OHdG formation levels in DNA and rise in gene mutations at higher doses, followed by induction of preneoplastic lesions at high doses. The primary importance of formation of DNA adducts by genotoxic chemicals, which contributes to the initiation stage of carcinogenesis is pointed out. Furthermore, formation of oxidative stress might be a secondary mechanism for the carcinogenicities of genotoxic carcinogens which is likely to be implicated in promotion and progression stages of chemical carcinogenesis.

Conflict of Interest

The authors declare no conflict of interest.

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