SYMPOSIUM PRESENTATION

Carcinogenic Risk Assessment: Are There Dose Thresholds for Carcinogens?

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Abstract

While it has been generally accepted that genotoxic carcinogens have no dose threshold for their carcinogenic potential, there is increasing evidence that very low doses in fact are incapable of inducing tumours or preneoplastic lesions. Thus not only so-called epigenetic 'non-genotoxic' compounds like phenobarbital and benzene hexachloride, but also unequivocally genotoxic carcinogens like the heterocyclic amines, 2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline and amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, and the nitrosamines diethylnitrosamine, and dimethylnitrosamine, may exhibit a practical dose threshold below which they do not induce histopathologically assessable lesions. Some form of physiological adaptation may thus be expected to occur in response to low doses of all types of DNA-damaging agents. With 'non-genotoxic' agents there may even be hormesis or paradoxical protection at very low dose.

Key Words: Dose dependence - carcinogenesis - threshold - genotoxic/non-genotoxic - hormesis

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Introduction

It has been generally accepted that genotoxic carcinogens have threshold in exerting carcinogenic potential for their risk assessment. On the other hand, non-genotoxic carcinogens have threshold. There is, however, a lack of data available for supporting these carcinogenic theories. Therefore, it is very important to resolve this point from the viewpoint of cancer risk assessment and management.

In this paper low dose carcinogenicities of both non-genotoxic carcinogens and genotoxic ones are reported from viewpoint of the carcinogenic mechanism using medium-term boaassay for carcinogens.

Low Dose Carcinogenicity of Non-genotoxic Carcinogens

Phenobarbital, used as a sedative, is non-genotoxic and carcinogenic to rodents. Figure 1 shows the protocol of the rat liver medium-term bioassay (Ito's test) employedin the hepatocarcinogenicity study of phenobarbital. Male F344, 6-week-old, rats were initiated with diethyl-nitrosamine (DEN) at 200 mg/kg b. w. and after 2 week administered phenobarbital at various doses (0,1 to 500 ppm in diet) for 6 weeks. The animals were subjected two-third partial hepatectomy at week 3. Numbers and areas of GST-P positive foci of more than 0.2 mm in diameter in the liver were measured using an image analyzer.

Numbers and areas of GST-P positive foci were significantly increased by treatment with phenobabital at doses of 60 ppm and over (Figure 2). Interestingly treatments with low doses tended to decrease development of GST-P positive foci, and particularly their numbers at 2 ppm and the areas at 1 and 2 ppm were significantly lowered. Thus the induction curve of GST-P positive foci showed a J-shape, indicating a hormesis phenomenon. Additional data showed phenobarbital treatment at 2 ppm

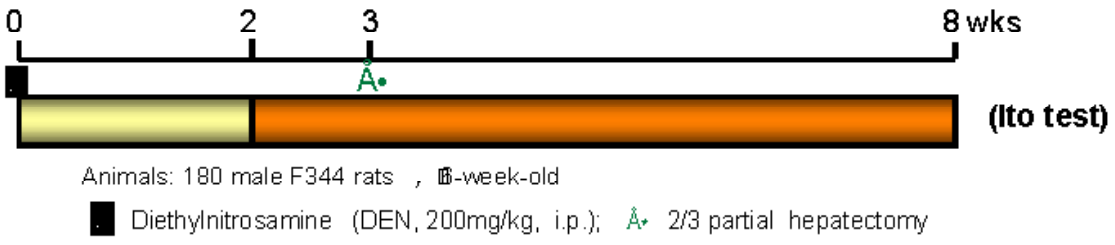

Figure 1. Experimental Protocol for the Ito Test of Phenobarbital at Low Doses with GST-P + Foci as End-points

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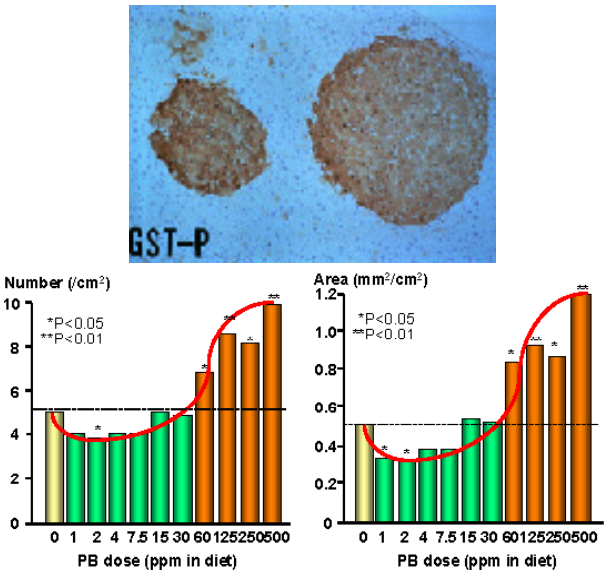

Figure 2. Quantitative Data for GST-P Positive Foci

to inhibit cell proliferation within GST-P positive foci in clear contrast to the enhancement observed with 500 ppm (Kinoshita et al., 2003)..

The α-isomer of benzene hexachloride (α-BHC) also showed hormesis for inducing GST-P foci in the rat liver, indicating the existence of a carcinogenic threshold (Puatanachokchai et al., 2006).

Low Dose Carcinogenicity of Genotoxic Carcinogens

Hepatocarcinognicity of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)

MeIQx is one of food-derived, heterocyclic amines which exert hepatocarcinogenicity in rats. A total of 885 male F344 rats, 21-days-old at the start of experiment, were administered MeIQx at doses 0, 0.001 to 100 ppm in diet for a maximum of 32 weeks (Fukushima et al., 2002). At week 16, numbers of GST-P positive foci (more than 2 cells) in the liver were similar among 0 to 1 ppm MeIQx groups, while MeIQx at a dose of 10 ppm showed a tendency for increase and 100 ppm significantly increased their numbers (Figure 3). The numbers of GST-P positive foci at week 32 showed similar results. MeIQx-DNA adduct formation in the liver of rats treated with MeIQx for 4 weeks demonstrated a linear relationship with 0.01 to 100 ppm detected (Figure 3). Formation levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the liver were linearly elevated from 1 ppm MeIQx at week 4 (Figure 3).

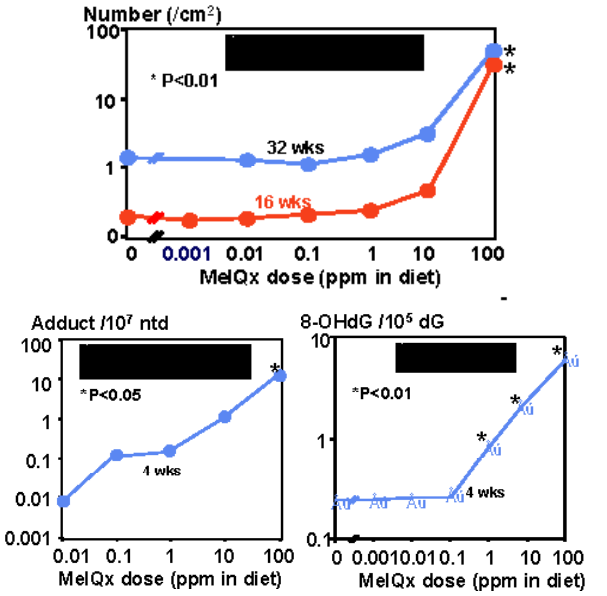

Figure 3. Quantitative Data for GST-P⁺ Foci, MeIQx-DNA Adducts and 8-OHdG

In Big Blue transgenic rat mutagenesis assay, MeIQx at low doses was found not induce lacI gene mutations in the liver at 16 week treatment and this correlates with non-induction of GST-P positive foci. However, the dose of MeIQx at which in vivo mutagenicity was significantly increased was lower than for GST-P positive foci (Hoshi et al., 2004). With MeIQx initiation at various doses followed by administration of phenobarbital, no induction of GST-P positive foci was noted at doses of 0.0001 to 1 ppm. MeIQx at doses of 10 and 100 ppm significantly increased development of GST-P positive foci. Thus the result implies no effect dose for initiation of hepatocarcinogenicity with MeIQx (Fukushima et al., 2003).

Levels of MeIQx-DNA adducts linearly increase from very low doses, and then, in order, curves for 8-OHdG formation levels and lacI gene mutation, initiation and then GST-P positive foci, preneoplatic lesions develop from different baseline control levels. These results indicate that MeIQx has different no-effect levels for induction of carcinogenesis-relating changes in rat liver.

Hepatocarcinogenicity of N-nitroso compounds, diethylnitrosamine (DEN) and dimethylnitrosamine (DMN)

Male, 21-day-old, F344 rats (about 1950) received DEN at various doses of 0, 0.0001 to 1 ppm in drinking water for 16 weeks. Numbers of GST-P positive foci in the liver in the groups at 0. 01 ppm and less were not different from 0 ppm control group. Treatment with DEN at doses of 0.1 and 1 ppm significantly increased GST-P positive foci development (Fukushima et al., 2002).

For DMN hepatocarcinogenicity, numbers of GST-P positive foci in groups at 0.001 to 0.1 ppm did not differ from 0 ppm control group, in contrast to the significant increase in groups treated with 1 and 10 ppm (Fukushima et al., 2005).

Large intestine carcinogenicity of amino-1-methyl-6-phenylimidazo[4,5-b]quinoxaline (PhIP)

A total of 1920 male F344 rats, 6-week-old received PhIP at various doses of 0, 0.001 to 400 ppm in diet for 16 weeks. PhIP-DNA adduct formation was significantly increased at doses of 0.01 ppm and over. Development of aberrant crypt foci which are surrogate markers of rat colon carcinogenicity was significantly increased at doses of 50 ppm and over (see Figure 4) (Fukushima et al., 2004).

In another study, male 6-week-old, F344 rats (20 in each group) were given azoxymethane treatment (15 mg/kg b.w., s.c., once a week, 2 times) and 2 weeks later,

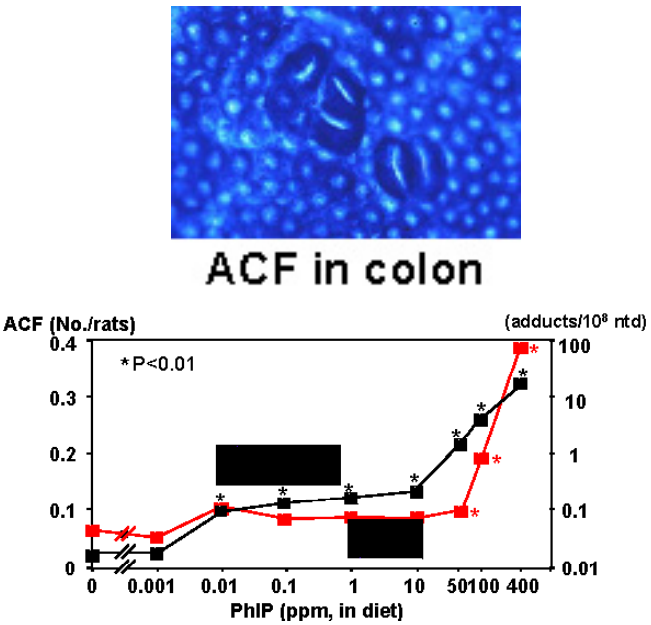

Figure 4. Quantitative Data for PhIP-DNA Adducts and ACF

treated with PhIP at various doses of 0, 0.001 to 200 ppm in diet for 34 weeks (Doi et al., 2005). As results at week 36, incidences and numbers of tumors (adenomas and / or carcinomas) in the large intestine were significantly increased by treatments with doses of 50 and 200 ppm.

Conclusion

With non-genotoxic carcinogens such as phenobarbital and a-BHC hormesis phenomenon (a J curve shape for dose dependence) appears to occur with regard tor induction of GST-P positive foci in rat liver. This result demonstrates that there is existence of threshold in rat hepatocarcinogenicity of phenobarbital. Even with genotoxic carcinogens like MeIQx, DEN and DMN for hepatocarcinogenicity and PhIP for large intestine carcinogenicity, a carcinogenic threshold, at least practically with reference to generation of histopathologically or immunohistochemically demonstrable lesions also exists. Adaptation may be expected to occur in response to low doses of all types of DNA-damaging agents.

References

Doi K, Wanibuchi H, Salim EI, et al (2005). Lack of large intestinal carcinogenicity of amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine at low doses in rats initiated with azoxymethane. *Int J Cancer*, **115**, 870-8.

Fukushima S, Kinoshita A, Puatanachokchai R, et al (2005). Hormesis and dose-response-mediated mechanisms in carcinogenesis: evidence for a threshold in carcinogenicity of non-genotoxic carcinogens. *Carcinogenesis*, **26**, 1835-45.

Fukushima S, Moore MA, Tsuda H (2003). Appropriate models and an understanding of carcinogenic mechanisms--requirements for hazard risk assessment. *Asian Pac J Cancer Prev*, **4**, 75-8.

Fukushima S, Wanibuchi H, Morimura K, et al (2005). Lack of potential of low dose N-nitrosodimethylamine to induce preneoplastic lesions, glutathione S-transferase placental form-positive foci, in rat liver. *Cancer Lett*, **222**, 11-5.

Fukushima S, Wanibuchi H, Morimura K, et al (2002). Lack of a dose-response relationship for carcinogenicity in the rat liver with low doses of 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline or N-nitrosodiethylamine. *Jpn J Cancer Res*, **93**, 1076-82.

Fukushima S, Wanibuchi H, Morimura K, et al (2004). Existence of a threshold for induction of aberrant crypt foci in the ratcolon with low doses of 2-amino-1-methyl-6-phenolimidazo[4,5-b]pyridine. *Toxicol Sci*, **80**, 109-14.

Hoshi M, Morimura K, Wanibuchi H, et al (2004). No-observed effect levels for carcinogenicity and for in vivo mutagenicity of a genotoxic carcinogen. *Toxicol Sci*, **81**, 273-9.

Ito N, Tamano S, Shirai T (2003). A medium-term bioassay for rapid in vivo detection of carcinogenic potential of chemicals. *Cancer Sci*, **94**, 3-8.

Kinoshita A, Wanibuchi H, Morimura K, et al (2003). Phenobarbital at low dose exerts hormesis in rat hepatocarcinogenesis by reducing oxidative DNA damage, altering cell proliferation, apoptosis and gene expression. *Carcinogenesis*, **24**, 1389-99.

Kitano M, Ichihara T, Matsuda T, et al (1998). Presence of a threshold for promoting effects of phenobarbital on diethylnitrosamine-induced hepatic foci in the rat. *Carcinogenesis*, **19**, 1475-80.a

Masuda C, Wanibuchi H, Otori K, et al (2001). Presence of a no-observed effect level for enhancing effects of the α-isomer of benzene hexachloride (α-BHC) on development of diethylnitrosamine-initiated hepatic foci in rats. *Cancer Lett*, **163**, 179-85.

Puatanachokchai R, Morimura K, Wanibuchi H, et al (2006). Alpha-benzene hexachloride exerts hormesis in preneoplastic lesion formation of rat hepatocarcinogenesis with the possible role for hepatic detoxifying enzymes. *Cancer Lett*, **240**, 102-13.