Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 231 (2006) 304–308
CANCER Letters
www.elsevier.com/locate/canlet

Existence of no-observed effect levels for 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline on hepatic preneoplastic lesion development in BN rats

Min Wei[a], Taka-aki Hori[b], Toshio Ichihara[a], Hideki Wanibuchi[a], Keiichirou Morimura[a], Jin Seok Kang[a], Rawiwan Puatanachokchai[a], Shoji Fukushima[a,*]

[a]*Department of Pathology, Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan*
[b]*Taishou Hospital, 5-5-16 Sankenya-higashi, Taishou-ku, Osaka 551-0002, Japan*

Received 21 December 2004; accepted 10 February 2005

Abstract

There is increasing evidence that dose–response curve of genotoxic carcinogen is nonlinear and a practical threshold dose exists. However, little is known about differences in the dose–response relationship of genotoxic carcinogen among different strain rats. Herein, we showed that low doses of genotoxic carcinogen 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx) had no effects on induction of liver glutathione S-transferase placental form (GST-P)-positive foci in both BN and F344 rats, and therefore demonstrated the existence of no-observed effect level for hepatocarcinogenicity of this genotoxic carcinogen irrespective of strains. These findings further support our notion that a practical threshold dose for MeIQx hepatocarcinogenicity exists in rats.

Keywords: 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Cancer risk assessment; Hepatocarcinogenesis; No-observed effect level; Strain differences

1. Introduction

Exposure to environmental carcinogens is one of significant causes of human cancers. Some of environmental carcinogens cannot be completely eliminated, therefore, it is very important to assess and manage the potential risks associated with human exposure to these agents. Dose–response assessment

Abbreviations 2-AFF; 2-acetylaminofluorene; DEN; diethylnitrosamine; GST-P; glutathione S-transferase placental form; MeIQx; 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline; NOEL; no-observed effect level.

* Corresponding author. Tel.: +81 6 6645 3735; fax: +81 6 6646 3093.
E-mail address: fukuchan@med.osaka-cu.ac.jp (S. Fukushima).

0304-3835/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2005.02.029

defines the relationship between the dose of an agent and the probability of induction of a carcinogenic effect, and is one of the most important components of carcinogen risk assessment. The dose–response relationship for genotoxic carcinogens is generally assumed to be linear without a threshold dose below which carcinogenic effects are absent, meaning that genotoxic carcinogens may pose some risk at any level of exposure [1–3], although there is no definitive experimental evidence to support this suggestion.

2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), a heterocyclic amine detected in the cooked meat and fish, is a potent genotoxic carcinogen [4,5]. MeIQx induced hepatocellular carcinoma in male F344 rats at high doses [6,7]. However, our recent low-dose studies showed the existence of no-observed effect levels (NOEL) for MeIQx on induction of glutathione S-transferase placental form (GST-P)-positive foci in the livers of F344 rats [8,9]. GST-P-positive foci is a well-established preneoplastic lesion in the liver of rat and has been accepted as a marker for assessing carcinogenic potential in the liver [10]. Moreover, GST-P-positive foci in the liver has been suggested to be a useful end-point marker in assessment of carcinogenic effects of environmentally relevant concentrations of liver carcinogens [11]. More recently, we found that low doses of MeIQx did not increase mutation frequencies in rat livers and the dose–response curve for mutation frequency is nonlinear in an in vivo mutagenicity assay using male Big Blue rats with genetic background of F344 [12]. These findings argue against the linear no-threshold risk assessment model for genotoxic carcinogens and indicate that there is a practical threshold for hepatocarcinogenetic effects of MeIQx.

Given susceptibility to hepatocarcinogenesis varies considerably among different strains of rat [13], strain differences may exist in the dose–response curve. All above studies on MeIQx were conducted in F344 rat and little is known about the effects of low doses of this carcinogen on other strain rats. The major purpose of the present study is to determine whether the NOELs for MeIQx on induction of GST-P-positive foci exist in BN rats. We also compared the susceptibility of BN and F344 rats to carcinogenetic effects of MeIQx.

2. Materials and methods

2.1. Animals

One-hundred and eighty male BN rats and 180 male F344 rats, 20-days old, were obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan). The animals were housed in polycarbonate cages (5/cage) in a room with a targeted temperature of 23 ± 2 °C, humidity of $55 \pm 5\%$, and a 12 h light/dark cycle and ad libitum access to food and tap water.

2.2. Chemical and diets

MeIQx (purity, 99.9%) was purchased from Nard Institute, Nishinomiya, Japan. Basal diet (powdered MF) and MeIQx diets were prepared at Oriental Yeast Co. Tokyo, Japan, and concentrations of MeIQx in the diets were confirmed by HPLC. The lowest level of MeIQx fed in the diet was 0.1 ppm based on previous experiments showing this dose had no effect on GST-P-positive foci development in F344 rats [8].

2.3. Experimental procedures

At 21 days of age, BN and F344 rats were randomized into groups of 30 rats each. As shown in Table 1, BN rats (groups 1–6) and Fisher rats (groups 7–12) were fed diets containing 0, 0.1, 1, 5, 10, or 100 ppm of MeIQx. Body weight, water and food consumption were measured weekly. All rats were sacrificed under ester anesthesia after 16 weeks of treatment for examination of GST-P-positive foci in the liver. Briefly, livers were excised, weighed and 3 slices each from the left lateral, medial, and right lateral lobes were fixed in 10% phosphate-buffered formalin, embedded in paraffin for immunohistochemical examination of GST-P-positive foci. Anti-rat GST-P polyclonal antibody (Medical and Biological Laboratories Co., Ltd.) were used for immunohistochemical staining of GST-P, as described previously [14]. GST-P hepatocellular foci comprised 2 and more cells were counted under a light microscope [8]. Total areas of livers were measured using a color image processor (IPAP, Sumica Technos, Osaka, Japan), and then the numbers of the foci per square centimetre of liver tissue were calculated.